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Title:
Investigating Offspring Mental Health Outcomes Associated with Maternal Prenatal Alcohol Use

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September 2019

School of Psychological Science

A dissertation submitted to the University of Bristol in accordance with the requirements for award of the degree of Doctor of Philosophy in the Faculty of Life Sciences

Word count: 43,207

Abstract

Maternal prenatal alcohol use is associated with a range of harms in offspring, particularly for high levels of alcohol exposure. Yet prenatal alcohol exposure (PAE) is still common, particularly at low levels of exposure. The effects of low to moderate alcohol exposure during pregnancy are variable with complex aetiologies and often studied within younger offspring age groups. In the studies reported in this thesis, I investigated if PAE was associated with offspring mental health, particularly for internalising disorders within late adolescence, assessing if any associations shown from previous literature for younger offspring ages may persist into adulthood.

This thesis applied different methods to explore the associations between maternal alcohol use in pregnancy and offspring mental health. I conducted a systematic review exploring the association between PAE and offspring mental health. I then applied a negative control analyses within a longitudinal birth cohort to investigate the association between PAE and offspring depression. Next, I explored the potential environmental influences of parental drinking after birth on offspring mental health. I then used a Phenome Wide Association Study (PheWAS) to investigate the effect of maternal and offspring genetic variants for increased alcohol use on a wide range of offspring mental health phenotypes across the phenome. Lastly, I used repeated measures to investigate associations between PAE and offspring trajectories of depression and latent classes of conduct disorder.

I found evidence of an association between increased maternal prenatal alcohol use and offspring mental health problems that suggested a causal effect. However, all associations were attenuated or removed entirely after adjustment for potential confounders, which may be causing a large part of the associations found. The findings of PAE still showing associations with mental health outcomes even during late adolescence, would suggest the associations previously seen within the younger developmental ages may indeed also be shown until early adulthood. Overall this thesis highlights the many problems encountered when investigating this topic, meaning inferring the causal nature of effect is problematic.

Acknowledgments

I would like to thank my supervisors Professor Marcus Munafò, Professor Nic Timpson and Dr Luisa Zuccolo for their support, guidance and feedback throughout this PhD. I am very grateful for the participants of ALSPAC who have helped make this research possible, and to the MRC IEU for funding my studentship. I owe a massive thank you to Sharen Hockey-O'Keefe, whose support for all things admin and finance have been invaluable.

I am very grateful to everyone in the Tobacco and Alcohol Research Group who have supported me within my research. In particular to my workstream leads Robyn and Hannah, who have helped make the transition from Psychology to Epidemiology slightly less daunting as I tackled new methods…and didn't judge me for my (I'm sure many) rogue questions along the way. To Angela Attwood, thank you for convincing me that it 'probably was a good idea' to go to this PhD interview 4 years ago. You always helped to remind me that I had worked hard for this, and ultimately am very capable, something that is sometimes easily forgotten when working in academia.

A massive thank you to my friends and family who have listened to me ramble on about PhD life over these last 4 years, I love you all and I don't know how I would have gotten here without you. Thank you to my fellow PhD students in 5 Priory Road, and in particular my office mates (and future dames) Emily and Rebecca. You made the tough times seem manageable and I'm so glad that we have completed this journey together. I'm very grateful to my friend Kathryn for everything you've done to support me over the years and given me the motivation to get through the more challenging days. To 'the originals' from Devon, I am so lucky to have had you and your encouragement in my life for all these years.

To my mum, you are forever in my corner and your unwavering support means the world. I would also like to thank my brothers who have both set the bar so very high. I look forward to you both addressing me as Doctor Easey from now on…

Authors declaration

I declare that the work in this dissertation was carried out in accordance with the requirements of the University's *Regulations and Code of Practice for Research Degree Programmes* and that it has not been submitted for any other academic award. Except where indicated by specific reference in the text, the work is the candidate's own work. Work done in collaboration with, or with the assistance of, others, is indicated as such. Any views expressed in the dissertation are those of the author.

SIGNED: DATE: 10/09/2019

Publications

***Easey, K. E.,** Timpson, N. J., & Munafo, M. R. (2020). 'Association of prenatal alcohol exposure and offspring depression: A negative control analysis of maternal and partner consumption.'. *Alcoholism: Clinical and Experimental Research.* In press.

***Easey, K. E.,** Dyer, M, Timpson, N & Munafo, M. (2019), 'Prenatal alcohol exposure and offspring mental health: A systematic review'. *Drug and Alcohol Dependence*. 197, 344-353.

Easey, K. E., Mars, B, Pearson, R, Heron, J & Gunnell, D. (2019), 'Association of birth order with adolescent mental health and suicide attempts: a population-based longitudinal study'. *European Child and Adolescent Psychiatry,* 28(8), 1079-1086.

Dyer, M, **Easey, K. E.,** Heron, J, Hickman, M & Munafo, M. (2019), 'Associations of child and adolescent anxiety with later alcohol use and disorders: a systematic review and meta-analysis of prospective cohort studies'. *Addiction,* 114(6), 968-982.

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Attwood, AS, **Easey, KE,** Dalili, MN, Skinner, AL, Woods, A, Crick, L, Ilett, E, Penton-Voak, IS & Munafò, MR. (2017). 'State anxiety and emotional face recognition in healthy volunteers'. *Royal Society Open Science,* 4(5).

Preprints:

***Easey, K. E.,** Timpson, N.J., Munafò, M. R. (2018). Association of intrauterine alcohol exposure and offspring depression: A negative control analysis of maternal and partner consumption. Preprint available on bioRxiv: https://doi.org/10.1101/307462.

*These publications were adapted to form Chapters 2 and 3, as well as sections within Chapter 1. Maddy Dyer performed a data check on the systematic review; Marcus

Munafò and Nicholas Timpson provided guidance and feedback on study design and manuscript preparation for both manuscripts.

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List of Abbreviations

AUD	Alcohol use disorder
AUDIT	Alcohol Use Disorders Identification Test
ADHD	Attention deficit hyperactivity disorder
ALSPAC	Avon Longitudinal Study of Parents and Children
BIC	Bayesian Information Criteria
CBCL	Child Behavioural Checklist
CIS-R	Clinical Interview Schedule-Revised
CI	Confidence interval
DoH	Department of Health
DAWBA	Development and Wellbeing Assessments
DOHaD	Developmental origins of health and disease
DICA	Diagnostic Interview for Children and Adolescence
EPDS	Edinburgh Postnatal Depression Scale
FASD	Fetal Alcohol Spectrum Disorder
FAS	Fetal Alcohol Syndrome
FIML	Full information maximum likelihood
GWAS	Genome Wide Association study
GMM	Growth Mixture Modelling
KSADS	Kiddie Schedule for Affective Disorders
LCA	Latent class analysis
LCGA	Latent class growth analysis
MDD	Major depressive disorder
MR	Mendelian Randomization
MICE	Multiple imputation by chained equation
NIMH DISC-IV	National Institute for Mental Health Computerized Diagnostic Interview Schedule for Children Version IV
OR	Odds Ratio
ODD	Oppositional defiant disorder
PheWAS	Phenome Wide Association Study
PRS	Polygenic risk score
PAF	Population-attributable fraction
PTSD	Post-Traumatic Stress disorder
PAE	Prenatal alcohol exposure
SMFQ	Short Mood and Feelings Questionnaire

SNPs	Single-nucleotide polymorphisms
SES	Socioeconomic status
SDQ	Strengths and Difficulties Questionnaire

Chapter 1 Introduction

Sections of this chapter have been published in the Journal of Drug and Alcohol Dependence: Easey, K. E., Dyer, M. L., Timpson, N. J., Munafò, M. R. (2019). Prenatal alcohol exposure and offspring mental health: A systematic review. *Drug Alcohol Depend.* 1(197), 344-353.

1.1 Thesis motivation

Before beginning this PhD, my research background was in psychology. I had gained previous experience in assessing and applying interventions to reduce psychological harm and I became interested in understanding the potential pathways to harm and particularly what mechanisms may be influencing these. I chose a research project using epidemiological methods, which help to identify the incidence and risk factors for events (such as health behaviours). Learning and applying epidemiological methods would allow me to explore these interests and ultimately develop a range of new skills.

1.2 Thesis overview

Within this thesis, I investigated the intergenerational relationship between maternal alcohol use during pregnancy on offspring mental health outcomes in late adolescence. A key component of this thesis is the use of triangulation, where multiple approaches and methodologies were used to investigate if maternal prenatal alcohol use was associated with offspring mental health outcomes. Having identified a gap in the literature, I focus mainly on internalising disorders as this outcome is less well researched compared to associations between maternal alcohol use and offspring externalising disorders.

This thesis begins with a systematic review summarising the available literature on maternal prenatal alcohol use and offspring internalising disorders and conduct problems (Chapter 2), providing an up to date and comprehensive understanding of the evidence currently available. In Chapter 3, I investigate the associations between both maternal and partner alcohol use during pregnancy and offspring depression at age 18 using a negative control design, to help understand the possible causal effects of prenatal alcohol exposure (PAE) on detrimental offspring outcomes. Chapter 4 moves away from only focusing on alcohol exposures during pregnancy and instead investigates the associations between (again maternal and partner) postnatal alcohol consumption, and offspring mental health. This chapter allows further investigation into the environmental influences of parental

alcohol use on offspring mental health, and the confounding structures shown for increased alcohol use. In Chapter 5 I utilise an emerging technique of a Phenome Wide Association Study (PheWAS). Here I investigate associations between maternal genetic variants known to influence alcohol consumption, and a wide range of mental health phenotypes (n = 90) and alcohol phenotypes (n = 22). This chapter investigates the effect of these genetic variants present in mothers on their own mental health phenotypes, as well as intergenerational effects on offspring phenotypes which could imply a causal pathway between maternal alcohol use itself and those phenotypes. Child's own genetic risk for increased alcohol use also was investigated with their own mental health phenotypes. The final empirical chapter (Chapter 6), investigates the association between maternal PAE on offspring depression and conduct problems using repeated measures collected during childhood and adolescence, which allows trajectory and latent class analyses. This chapter investigated patterns of association and how these may change over time. In the final chapter, I discuss the findings from each of these chapters and what they add to what is already known, as well as how – taken together – they help us understand how maternal PAE may be associated with offspring mental health problems.

1.3 Alcohol use in pregnancy

Maternal health behaviours during pregnancy, such as tobacco and alcohol use, are associated with adverse offspring health consequences (Kodituwakku & Kodituwakku, 2014; A. E. Taylor et al., 2017). Alcohol is a teratogen that directly crosses the placenta when alcohol is consumed during pregnancy and can cause the blood alcohol level of the developing fetus to reach maternal blood alcohol levels. Despite evidence of the harmful effects of alcohol use during pregnancy, it remains common, particularly at low levels (O'Keeffe et al., 2015). A recent study estimated the global prevalence of maternal alcohol use in pregnancy as 9.8% (Popova, Lange, Probst, Gmel, & Rehm, 2017). In the United Kingdom, these rates of alcohol use may in part be due to recommendations from health services during pregnancy. Before 2016, the recommended UK guidelines for alcohol consumption advised pregnant women to avoid drinking alcohol during the first trimester, and that alcohol consumption should not exceed 1-2 units, once or twice a week (NICE, 2008). This advice was updated in January 2016 to reflect research that had been undertaken in the field, and the Department of Health's (DoH) UK Chief Medical Officer released an update to this guidance advising that abstinence was the safest approach throughout pregnancy as well as when trying to conceive. This guidance reflected evidence of there being no safe level of alcohol use during pregnancy. This updated guidance seems aimed at mothers who may consume low to moderate alcohol amounts in

pregnancy, in line with the previous recommendations of two small glasses of wine per week not being harmful for a developing fetus. The updated DoH guidance for complete abstention from drinking alcohol during pregnancy is based on the precautionary principle, in the absence of solid or substantial evidence of harm for low doses of (PAE). This proposes that absence of evidence for harm does not provide evidence of absence (Mamluk et al., 2017).

Globally the guidance for drinking alcohol during pregnancy is also framed in varying ways, with some countries mentioning only the first trimester as being harmful, or aligned with the outdated UK advice of 1-2 units per week not being harmful (IARD, 2019). In addition, there is a lack of literature investigating the influence of father or partner alcohol use during pregnancy and its influence on offspring mental health, with previous research showing nearly 20 times more papers have been published using terms related to maternal influences, compared to paternal terms (Sharp, Lawlor, & Richardson, 2018). It is suggested that this is due to the underlying assumptions that it is only maternal exposures that are critical (Sharp et al., 2018; Sharp, Schellhas, Richardson, & Lawlor, 2019). I have also included partner alcohol use in certain chapters, to ascertain if any associations were due to maternal alcohol use alone, partner alcohol use, or shared confounding. Any reference to PAE within this thesis refers to maternal PAE unless otherwise stated.

1.4 Fetal alcohol syndrome and Fetal alcohol spectrum disorders

Heavy alcohol use has been evidenced to cause physical and cognitive impairments (Bille et al., 2007; Sayal, 2007; Walthall, O'Connor, & Paley, 2008), and Fetal Alcohol Syndrome (FAS) (Mukherjee, Hollins, & Turk, 2006). Heavy and binge drinking alcohol (4+ drinks on a single occasion) whilst pregnant has long been advised against. There is large field of evidence concerning FAS and Fetal Alcohol Spectrum disorders (FASD). FAS results from a child's prenatal exposure to alcohol during pregnancy and was first reported by Jones and colleagues in the 1970s (Jones & Smith, 1973; Jones, Smith, Ulleland, & Streissguth, 1973). Unborn babies are unable to process alcohol as well as the mother, and PAE can therefore disrupt healthy intrauterine development. The severity of symptoms experienced in offspring varies considerably but can include physical and behavioural problems. FASD however, is an umbrella diagnostic term describing the lifelong disability of physical and cognitive impairments as a result of prenatal alcohol exposure. FAS is distinct from FASD, due to the visible physical growth deficiencies, such as a thin upper lip, short palpebral fissure length and smooth philtrum in those diagnosed with FAS (Streissguth et al., 1991). In women who consume any

alcohol during pregnancy, it has been estimated that 1 in 13 will have offspring with FASD, and 1 in 67 will have FAS (Lange et al., 2017; Popova, Lange, Probst, Gmel, et al., 2017). Offspring exposed to the same amount of PAE do not experience the same detrimental outcomes in severity and presentation (Montag, 2016). It is also recognised that FASD may also be undiagnosed (Elgen, Bruaroy, & Laegreid, 2007) and offspring outcomes of lower IQ, coordination problems and hyperactivity for example, may be present yet not diagnosed as FASD. Ultimately, the missed diagnosis of FASD means offspring are not getting the help they may need from intervention services (Chasnoff, Wells, & King, 2015). Much evidence has shown an association with heavy PAE and problematic behaviour for both internalising and externalising disorders in offspring diagnosed with FASD (O'Connor & Paley, 2009; Tsang et al., 2016). A recent systematic review and meta-analysis investigating neurodevelopmental disorders in children diagnosed with FASD, found externalising disorders of attention deficit hyperactivity disorder (ADHD) to be the most common co-morbid disorder (52.9%), followed by oppositional defiant disorder (ODD) (12.9%) (Lange, Rehm, Anagnostou, & Popova, 2018).

A large amount of research has also shown alcohol use to be associated with externalising disorders in non FASD samples. This could be indicating that PAE may have an influence on externalising disorders even without the presence of FASD, or it could be representing further under diagnoses of FASD. Due to the large area of research that has been conducted investigating the influence of heavy alcohol use and children with known FASD's, I sought to avoid focusing on FAS/FASD within this thesis and aimed to investigate low to moderate PAE instead of heavy use. I have therefore avoided including known FASD samples in the included literature and analyses where possible. However, due to the likelihood of offspring being included who may have FASD as it is vastly undiagnosed, it is important to have outlined the prevalence and similarities in potential detrimental outcomes.

1.5 Low to moderate alcohol exposure in pregnancy

Low to moderate alcohol exposure during pregnancy seems to be the area that has limited evidence and conflicting conclusions regarding its harm, despite the recent updates to the DoH's advice now promoting abstinence compared to the previous guidance of 1-2 units of alcohol once or twice per week not being likely to cause harm. Whether such light to moderate alcohol use during pregnancy may affect offspring outcomes is less clear. A recent meta-analysis found that only a small number of prospective studies have investigated the association of light to moderate maternal

alcohol use in pregnancy with offspring outcomes (Mamluk et al., 2017). This meta-analysis focused mainly on pregnancy outcomes such as gestational diabetes, and childhood outcomes that have been linked to FAS, such as behavioural problems and cognitive impairment. The authors describe the lack of evidence for either a harmful effect, or for a safe level of intrauterine alcohol exposure and highlight the poor quantity and quality of contributing studies. Such findings demonstrate the need for further research into low to moderate PAE through careful study design investigating the potential causal influence. A review by O'Leary and Bower in 2012 highlighted the strength of evidence available from low levels of PAE as weak, and that the associations found both for detrimental and 'protective' influences of light PAE are likely due to residual confounding influences and misclassification of exposures (O'Leary & Bower, 2012). However, the authors also describe more recent studies having shown 1-2 alcoholic drinks during pregnancy once or twice a week (in line with recommended government guidelines at the time of publication) being associated with neurodevelopmental problems, and suggested there may be a small margin of detecting the potential harm of low PAE.

1.6 Externalising disorders and behavioural problems

The relationship between PAE and offspring externalising problems has often been investigated, perhaps due to the high rates of behavioural problems associated with high levels of prenatal alcohol use and FASD (Brown et al., 1991; Lange et al., 2018; O'Leary et al., 2009). PAE has been linked with adverse offspring behaviour not just for high levels of alcohol use, as expected from FASD research, but also low to moderate PAE. Previous research has even suggested differences in the subtypes of offspring behavioural outcomes that may be influenced as being dependent on the amount of PAE (Sood et al., 2001). Sood and colleagues (2001) found a dose-response pattern between PAE and offspring externalising behaviour. Higher mean scores were observed for low amounts of PAE and offspring externalising and aggressive behaviours at age 6, and higher mean scores for total problem scores and delinquent behaviour were only shown for moderate and heavy PAE. Such findings evidence a difference in which offspring behaviours varying levels of alcohol use can influence. Their study found associations persisted after adjustment for a range of confounding factors however, the authors note the sample was largely represented by socially disadvantaged families. Their findings may therefore have been influenced by residual confounding.

PAE has often been associated with offspring attention problems (such as hyperactivity, ADHD), and conduct disorder (Larkby, Goldschmidt, Hanusa, & Day,

2011). Disney and colleagues sought to test if the association of PAE and offspring conduct problems in adolescence was mediated by parental externalising disorders, as this may be reflective of being raised in an adverse environment (Disney, Iacono, McGue, Tully, & Legrand, 2008). Comparable to previous research they also showed PAE contributed to an increased risk of offspring conduct problem symptoms at age 17, and these associations persisted even after controlling for potential confounders and parental externalising disorders. The strength of these associations even at a later offspring age would suggest that the detrimental influence of PAE on offspring behavioural problems may continue into adulthood. However, in this study the alcohol exposure was derived from asking how much alcohol mothers drank during gestation, and mothers consuming one or more alcoholic drinks per week was classed as having PAE. This derivation therefore does not measure varying levels of alcohol exposure. The authors did control for alcohol dependence, yet their findings still may be representative of heavier drinkers within the sample. D'Onofrio and colleagues also sought to investigate the potential causal factors influencing associations between PAE and offspring externalising problems, utilising sibling controls (D'Onofrio et al., 2007). Again, the authors found PAE to be associated with an increased risk of conduct problems in unrelated offspring. However, when they compared this within siblings, they found offspring who were more exposed to PAE did not have greater levels of conduct problems compared to siblings with less PAE. D'Onofrio and colleagues suggest the association between PAE and offspring externalising disorders are likely to be due to other confounding factors related to increased maternal alcohol use.

1.7 Internalising disorders

Much of the research published to date has focused on physical or behavioural outcomes, with the influence PAE has on internalising disorders being less clear. Exposures during pregnancy have however been shown to represent risk factors for adverse internalising mental health outcomes also, reflecting the fetal environment, genetic contributions and prenatal environmental effects (Schlotz & Phillips, 2009). Similar to research conducted on externalising problems, increased maternal PAE has been associated with detrimental offspring mental health. However, again, the evidence that is available often focuses only on moderate to heavy PAE (Fryer, McGee, Matt, Riley, & Mattson, 2007), and not light to moderate.

O'Connor and Kasari published the first study to investigate the influence of PAE on offspring depression. They measured average maternal alcohol consumption retrospectively one year after birth and found increased PAE was associated with

increased offspring depressive symptoms at age 5-6 years (O'Connor & C Kasari, 2000). Future studies sought to assess the influence of light PAE on offspring mental health. Sayal and colleagues found that mothers consuming less than one alcoholic drink per week during the first trimester was associated with adverse mental health outcomes for female offspring at 8 to 9 years of age (Sayal, Heron, Golding, & Emond, 2007). A follow up study measured mental health outcomes within older age groups and found drinking less than one alcoholic drink per week during the first trimester to be weakly associated with offspring total problems in females after adjustment (Sayal et al., 2013). These differences between studies could however be due to the longer follow up from exposure to outcome age, and any associations being weakened because of cohort attrition. However, it should be noted that these findings are from total problem scores as measured by the Strengths and Difficulties Questionnaire (SDQ) (Goodman, 1997, 2001) which consists of externalising disorder subscales also.

Light alcohol use in pregnancy has also been reported to be associated with improved offspring outcomes (i.e., appears protective) (Kelly et al., 2012; Robinson et al., 2010). Kelly and colleagues (2012) found that drinking 1-2 units of alcohol in pregnancy was associated with higher cognitive abilities in male offspring at age five, with worse offspring outcomes observed at either end of consumption levels for both abstainers or heavy drinkers. The authors found drinking to be socially patterned and suggest that these findings may be due to residual confounding, as mothers who reported light alcohol use were more likely to be from higher income households and with higher levels of education. Robinson and colleagues also found evidence to suggest that low PAE was associated with decreased problem scores in offspring internalising problems across a 14 year follow up (Robinson et al., 2010). However, the authors note that this finding may not be due to sample attrition within the cohort, but instead due to the over representation of socially disadvantaged families included in the analysis. They concluded that light to moderate PAE is not a risk factor for the offspring outcomes measured. Kelly et al reviewed the literature within the negative offspring psychiatric outcomes and highlighted the changes in social behaviour that can occur with alcohol consumption, and suggested that offspring who may be exposed to alcohol prenatally may have differences in the way their social behaviour was grounded in early life (Kelly, Day, & Streissguth, 2000). Associations found may therefore still be due to residual confounding which may have influenced early life behaviours.

There are also important methodological differences across studies, such as the way that mental health outcomes are measured. Some studies report only a total internalising or externalising disorder score, without showing how the subscales of each item (such as anxiety or depression) contribute individually (Robinson et al., 2010).

Without a standard measure used across studies, differences in methodology can introduce substantial heterogeneity and mean that comparison or replication of findings becomes problematic. Of the research that is available, many studies report outcomes for young age groups, showing the impact prenatal alcohol exposure may have during the developmental stages of childhood only. However, it is less clear how prenatal alcohol exposure may affect offspring mental health as the child becomes older, and if any associations shown at earlier ages persist into adulthood. Often early childhood measures of offspring mental health are given by parental self-report, instead of by the child themselves which could affect the reliability of these measures.

1.8 Factors influencing the strength of evidence

What is apparent within the literature is a lack of agreement of what level of alcohol exposure contributes towards varying forms of offspring risk. O'Leary and Bower sought to review the current research from systematic reviews and meta-analyses already conducted which investigated low to moderate PAE (O'Leary & Bower, 2012). The authors highlight vast methodological weaknesses across studies which may account for differences in association, such as misclassification of exposures and outcomes, and confounding influences. Conducting research within the area of PAE and offspring mental health outcomes requires suitable longitudinal data across repeated timepoints for exposure and outcome. Many of the studies investigating this area utilise longitudinal cohorts, in which the same group of individuals are followed over time and provide repeated observations. This type of data collection is advantageous as it allows measurement of exposures and outcomes without manipulating participants. However, often the most appropriate exposure or outcome measure required to address this question is not available due to the retrospective nature of these studies.

Misclassification of alcohol use is a major problem when interpreting findings from studies investigating PAE (O'Leary et al., 2010), as most studies record alcohol use via different measures. Some studies ask only if alcohol was consumed during pregnancy (yes/no), with no separation for the frequency or amount of alcohol consumed (Bada et al., 2007; Disney et al., 2008; Fryer et al., 2007) meaning we cannot study any potential dose-response effects. Of the studies that do expand their measurement to include different amounts of PAE, many include categories of "low, moderate or heavy" amounts. However, what amount of alcohol is categorised as low, medium or heavy varies greatly between studies. This may mean that what one study classes as moderate alcohol exposure, another may class as low. Therefore, it is hard to discern whether any

associations (or their absence) are due only to the differences in alcohol measurements used. It also means meta-analyses and comparison between studies is problematic.

Differences are also apparent in which trimester PAE is measured. With some studies asking mothers to report on the entire pregnancy (Niclasen, Andersen, Strandberg-Larsen, & Teasdale, 2014a; O'Connor & Kasari, 2000) and some focusing only on early or late trimesters (Day, Helsel, Sonon, & Goldschmidt, 2013; Niclasen, Andersen, et al., 2014a; O'Connor, 2001). The early stages of pregnancy (first trimester) have often been reported to be the most sensitive to teratogenic influences of PAE. O'Connor found alcohol exposure as early as 8 weeks gestation to be the most sensitive period during pregnancy (O'Connor, 2014). Such findings are likely why many studies focus on the first trimester. Mothers may not be aware of their pregnancy in the early stages, which means they may be inadvertently exposing the developing fetus to high levels of alcohol.

As briefly mentioned, many of the findings in previous research may be influenced by the confounding structures within the samples used. A recent meta-analysis has collated the research conducted on PAE and offspring outcomes, finding both internalising and externalising disorders to be associated with PAE (Khoury, Jamieson, & Milligan, 2018). However, the authors also highlighted how the strength of association is often moderated by distinct confounding characteristics within studies such as socio-economic status, age, the amount of alcohol consumed, and the type of offspring outcomes included. For example, a higher risk of negative offspring outcomes/FASD has been shown for families from lower socioeconomic status (SES), lower educational attainment and older mothers (Cannon et al., 2012; Kvigne et al., 2003; May et al., 2004). These findings may not be due to an increased age, but due to ingrained alcohol behaviours developed across the life course. Older women have been shown to consume alcohol more frequently, with younger women drinking less frequently, but consuming higher amounts on the occasions they did drink alcohol (Britton, Ben-Shlomo, Benzeval, Kuh, & Bell, 2015). If these patterns persist during pregnancy, it may suggest that older pregnant mothers consume alcohol more frequently, therefore exposing the developing fetus to alcohol more often than younger mothers. This highlights the problem of confounding when investigating alcohol exposures, and how individuals at risk may potentially be protected by social advantage within older mothers.

Associations between PAE and offspring mental health could be shown due to confounding influences, both environmentally and genetically (Thapar & Rutter, 2009). Studies that collect data using participants from higher socio-economic families may without meaning to, be introducing bias into their data, as individuals may have a social advantage in comparison to those from a lower socio-economic background. Social support during pregnancy has been linked with lower alcohol use during pregnancy in

European and American samples (McQuire, Daniel, Hurt, Kemp, & Paranjothy, 2019), this could perhaps be indicative that women who continue to consume higher levels of alcohol whilst pregnant may be exposed to greater stress. Lack of social support, increased stress and depression during pregnancy has been shown to negatively influence offspring outcomes (Elsenbruch et al., 2007; O'Connor, Heron, & Glover, 2002). Any findings for negative offspring outcomes may therefore be moderated by such confounding influences. Most studies will ultimately seek to test the influence of such confounders by including an adjusted analysis. However, exactly which confounders are chosen to be included in analysis models varies greatly between studies (Easey, Dyer, Timpson, & Munafò, 2019), which again makes comparisons between studies problematic. Being able to control for potential confounders is also only as successful as the measurement of these items within each study. If a study does not record a suitable measure, it limits any adjustment for confounding influences which may be driving associations.

The age that offspring outcomes are measured is also something that is varied across studies, perhaps again due to the availability of longitudinal data. The majority of studies seem to assess offspring mental health outcomes in early childhood (Alvik, Aalen, & Lindemann, 2013; Bada et al., 2007; Sood et al., 2001). Very few have investigated the long-term influences (Day et al., 2013; Disney et al., 2008). Studies that have focused on developmental ages can show proximal influences, but not distal. This means we are unable to say with certainty that any associations shown for detrimental offspring outcomes in early childhood are also present in early adulthood. The literature is lacking evidence of how PAE can influence how mental health outcomes may change over time, and the patterns such outcomes may take. If PAE is indeed associated with detrimental offspring outcomes, and as discussed mothers are still consuming alcohol whilst pregnant, finding critical stages of life where interventions may be implemented is very important.

1.9 Summary

In summary, the literature currently available on associations between PAE and offspring mental health outcomes has shown variable findings and complex aetiologies, particularly for low to moderate PAE. However, this variability could be due to differences in methodologies across studies, such as exposure and outcome measurement and definition. More work is needed focusing on the same alcohol exposures measured at the same gestational period across studies. Having reviewed some of the already available research investigating associations between PAE and offspring mental health and considering the limitations within what has already been conducted, I seek to develop the

available literature. Within this thesis, I will use the same measure of PAE across chapters where possible, in order to investigate the association between PAE and offspring mental health; with a focus (albeit not exclusive) on internalising disorders. Much of the previous research has also focused on higher levels of alcohol exposure as opposed to low levels, and externalising as opposed to internalising disorders in offspring. Within this thesis I therefore address these less researched areas by investigating the associations between maternal PAE and offspring mental health in late adolescence. A key component of this thesis is the use of triangulation, by applying multiple approaches and methodologies to investigate offspring mental health outcomes associated with maternal prenatal alcohol use.

Chapter 2 Prenatal alcohol exposure and offspring mental health: A systematic review

A version of this systematic review has been published in the Journal of Drug and Alcohol Dependence: Easey, K. E., Dyer, M. L., Timpson, N. J., Munafò, M. R. (2019). Prenatal alcohol exposure and offspring mental health: A systematic review. *Drug and Alcohol Dependence*. 1(197), 344-353.

2.1 Background

As described in Chapter 1 there is evidence from longitudinal research to suggest there is an association between PAE and adverse physical health consequences in offspring (Bille et al., 2007; Larkby & Day, 1997). However, the literature is less clear on the association of alcohol use in pregnancy and offspring mental health, with studies evidencing both a detrimental (Alvik et al., 2013; Knopik, Heath, Bucholz, Madden, & Waldron, 2009; O'Leary et al., 2009) and 'protective' influence (Kelly et al., 2009; Robinson et al., 2010) of PAE. Much research is conducted towards investigating the influence of heavy PAE with fewer prospective studies investigating light to moderate PAE (Mamluk et al., 2017). The aim of this chapter was to systematically review the existing literature on PAE and offspring mental health outcomes, with a focus on internalising disorders. This review was exploratory and sought to investigate and describe any patterns of association previously reported for varying types of mental health. The aim of this chapter was to inform subsequent chapters and to help build upon and direct the research question of this thesis which investigated the influence of maternal PAE on offspring mental health outcomes.

2.2 Method

2.2.1 Selection strategy

This review was conducted according to PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-analyses) guidelines (Moher, Liberati, Tetzlaff, Altman, & Group, 2009), and was preregistered on the Open Science Framework (osf.io/yrn2r). Electronic databases (PsycINFO, PubMed and Web of Science) were searched until mid-March 2017 to identify English language publications.

Screening of study eligibility was conducted, and irrelevant articles excluded based on title and abstract (see section 2.2.2). All study types were included (e.g. case control, cross-sectional, cohort studies) if they met the inclusion criteria. Full-text articles were subsequently reviewed to determine eligibility, with reasons for exclusion

documented for each paper. I conducted all stages of the review, and a 10% check of all the articles found at each of these stages were completed by a second reviewer (MLD in the published manuscript), and any disagreements on eligibility were discussed and resolved by mutual consent.

2.2.2 Eligibility criteria

The search strategy included key words related to "pregnancy", "alcohol", and "mental health". The following search terms were used "mental health" OR depress* OR anxiety OR mood OR conduct OR internali?ing AND alcohol OR ethanol OR drink* AND pregnan* OR perinatal OR prenatal OR intrauterine OR utero OR f?etal OR gestation. At the initial stage of extraction, studies were excluded if they were review articles or animal studies. As the association between heavy drinking and FASD is well established, studies which only investigated known FASD samples were also not included. This was to further refine the review away from clinical diagnoses of FASD and potentially heavier alcohol exposures during pregnancy. Many of the FASD symptoms have a strong externalising component also, and this review sought to focus on the effects on internalising disorders. However, it is noted that FASD has high comorbidity with many conditions, including the internalising disorders that were focused on within this chapter (Popova et al., 2016). FASD is also known to be underdiagnosed, and therefore studies included within this review may still be representing offspring with undiagnosed FASD, despite efforts to limit this. Measures of maternal PAE during pregnancy (e.g. prenatally and not postnatally) were included only.

Any source of mental health measure was included (e.g., self-report or maternal report). Outcomes measured below the age of three were excluded also. As discussed in Chapter One much of the research conducted on the influence of PAE and offspring outcomes tends to have focused on birth outcomes and early developmental stages of childhood. I therefore sought to exclude earlier childhood outcomes (<3 years old), with a focus on childhood to adolescence and early adulthood, to summarise the literature already available across the life course to ascertain if any associations seen may be suggesting permanency.

Eligible studies were included if they contained the desired outcome and exposure variables within their data set, which meant the included studies were not always initially designed to investigate associations between prenatal alcohol exposure and offspring mental health.

2.2.3 Data extraction

Data were extracted on exposures, outcomes, study location, design, maternal age during pregnancy, offspring sex and age at outcome, to investigate alcohol use in pregnancy and offspring mental health outcomes, as well as confounders included in the most fully adjusted model within the study. A 100% check on the data extraction was also conducted by an independent researcher (MLD).

If studies reported multiple alcohol exposures from varying stages of pregnancy, the earliest time point was extracted. This inclusion was for consistency across studies, but also because previous research has shown maternal health behaviours in the earlier stages of gestation to have greater influence compared to later trimesters (Feldman et al., 2012). Where multiple alcohol exposure types (e.g., cumulative or binge drinking) were used, the cumulative alcohol amount was extracted. If studies reported mental health outcomes at multiple ages, results from the oldest age group were extracted because this review was aiming to access if any influences of PAE were long lasting across childhood and likely to be present in early adulthood. Fully adjusted results are presented when reported in studies. If included studies reported multiple mental health outcomes, the data were extracted separately for each outcome to investigate whether any subscale of mental health is most strongly associated with intrauterine alcohol exposure. Data from sensitivity and subgroup analyses, such as additionally splitting analyses by sex were not extracted to allow comparison between a greater number of studies.

2.2.4 Data Analysis

Within the pre-registered protocol, a meta-analysis was planned if deemed appropriate from the included studies. However, a meta-analysis was not conducted as there were substantial differences between studies in exposure measurement, time to follow up, location, covariates used, and prevalence of outcomes sampled. As a meta-analysis was not possible, I have instead presented an appraisal of the current literature, enabling the reader to be aware of the limitations in interpretation, and further provided suggestion for how future studies may improve the synthesis of evidence.

2.3 Results

2.3.1 Characteristics of included studies

The initial search identified 3,397 articles (after removal of duplicates), of which 65 were chosen for full text review after exclusion of irrelevant studies based on title, abstract and keywords. Of these, 32 did not meet inclusion criteria and were excluded

(see Figure 2.1). Thirty-three articles met the inclusion criteria, details of which are shown in Table 2.1. Six studies used a UK population, 17 USA, 5 Australian, 3 Scandinavian, 1 Canadian, and 1 Taiwanese. Details of excluded studies are shown in the appendices (Appendix 2.1).

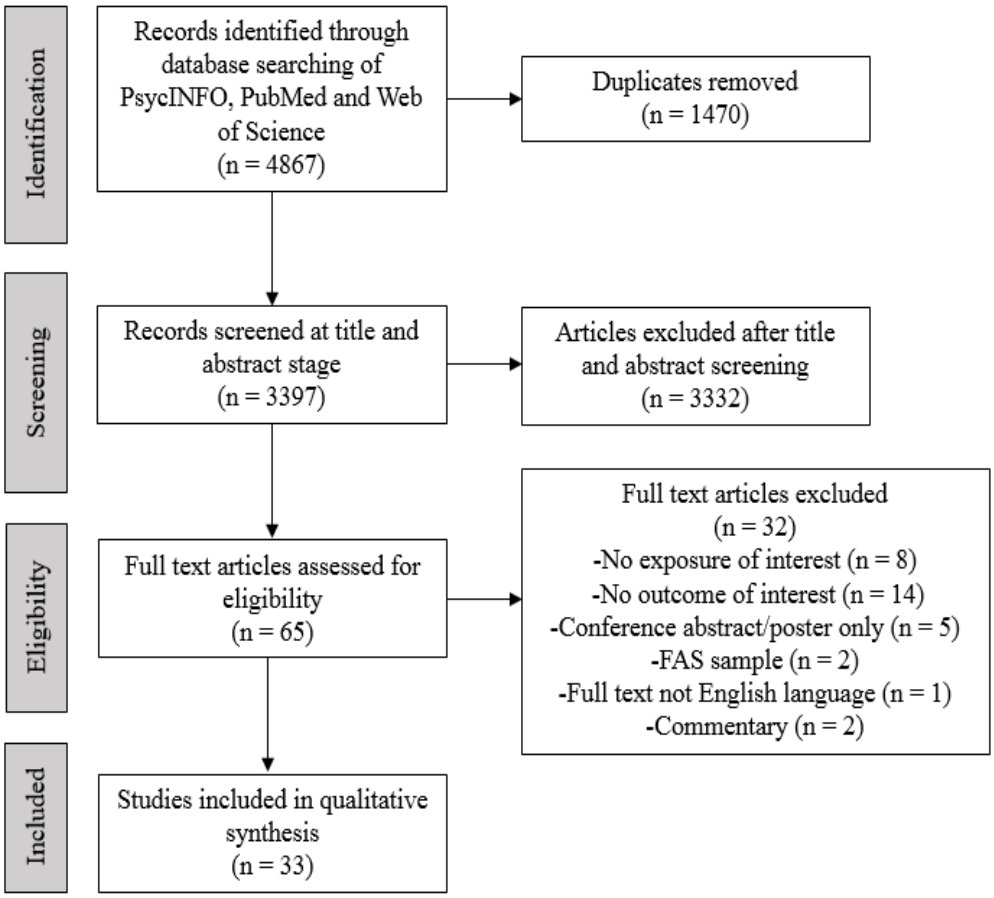

Figure 2.1: Flowchart of search strategy, reasons for exclusion and final included studies

2.3.2 Summary of results

Studies ranged in sample size from 41 to 37,315, and length of follow up from 3 to 26 years. Of the 33 included studies, 23 (70%) reported using male and female participants, 1 (3%) reported only using females and 9 (27%) did not report the sex of the participants.

The associations described refer to a positive association (e.g., intrauterine alcohol exposure was associated with increased depression) unless stated otherwise.

2.3.3 Assessment tools used

The exposure of prenatal alcohol use was measured using a binary or categorical measure for 30 of the 33 included studies. Of these, 4 used a binary exposure to measure alcohol consumption during pregnancy (yes/no). The remaining 26 studies all used varying categorical exposures, with different definitions of "low", "moderate" and "binge" alcohol exposure used between studies (see Table 2.1). Of the 3 studies that did use a continuous measure of drinking, all measured different types of alcohol exposure (e.g., average daily volume of alcohol, cumulative alcohol intake across pregnancy, maximum number of drinks per occasion). Of the 33 included studies, 27 measured PAE by self-report, 3 used measures of a documented history of alcohol exposure in utero, 2 used mixed reports of parent self-report as well as observed reports of alcohol exposure, and 1 study did not state how PAE was measured.

Ten studies used the Strengths and Difficulties Questionnaire (SDQ) (Goodman, 1997, 2001) as the primary measure of offspring mental health, 13 studies used the Child Behavioural Checklist (CBCL) (Achenbach, 1991), 3 used the Pictorial Depression Scale (O'Connor & C Kasari, 2000), 1 used the structured Clinical Interview for DSM-III R Personality disorders (Spitzer, Williams, Gibbon, & First, 1987), 1 used the Diagnostic Interview for Children and Adolescents (DICA) for telephone administration (Reich, 2000), 1 used the Diagnostic Interview Schedule for DSM-IV (Robins et al., 2000), 1 used the Kiddie Schedule for Affective Disorders (KSADS) (Chambers et al., 1985), 1 used the National Institute for Mental Health Computerized Diagnostic Interview Schedule for Children Version IV (NIMH DISC-IV) (Shaffer, Fisher, Lucas, Dulcan, & Schwab-Stone, 2000), 1 used both the KSADS and NIMH DISC-IV combined, and 1 study did not report the measure used.

Due to the different types of scales/measures used across studies, I categorised studies on the type of mental health outcome they reported measuring: Anxiety/depression (measures of anxiety, depression, withdrawn/depressed, generalised anxiety disorder, separation anxiety and major depression were combined due to the limited number of studies using each individual scale and their comorbidity), emotional problems, total internalising score, total problem score, and conduct disorder. The percentages of associations reported below are indicative of the total number of studies included within each outcome subscale.

The 33 studies included in this review included ten varying measures of assessing mental health, seven of which were used within only one study each. To aid interpretation of the literature, I sought to create a categorization system that captured every subscale

used by the studies in our review. This was guided by the Strengths and Difficulties Questionnaire and Child Behaviour Checklist, which was used for outcome measurement within the majority of studies (23/33; 67%). This was not an effort to generate a new categorization system, but to clarify the coverage of existing literature. Only select studies reported the "total scores" of either internalising or total problem scores and are reported in this review when available in each paper. Such total problem scores are derived from the individual mental health subscales also presented. However, description of both the total problem scores and individual subscales are given within this review to allow a more comprehensive overview of the findings reported.

Anxiety/depression

A total of 13 studies investigated the association of maternal PAE with subsequent offspring anxiety/depression. Of these studies, 9 (69%) found evidence to support a positive association of increased maternal PAE and increased offspring anxiety/depression (n = 41 to 1,327), and 4 (31%) found no evidence of an association (n = 11 to 321). Of the 9 studies reporting a positive association, 6 of these studies investigated a population with either low socioeconomic status (SES), or offspring with other presenting mental health problems such as attention deficit hyperactive disorder (ADHD). Of the 4 studies reporting no clear evidence of an association, 3 utilised a sample of offspring with a diagnosed mental health problem, or from a family with a history of having an alcohol problem. The remaining study that did not find an association had a small sample of only 11 mothers who consumed alcohol during pregnancy and may have been underpowered to detect an association.

Emotional problems

A total of 4 studies investigated the association of maternal PAE with subsequent offspring emotional problems. Of these studies, 2 (50%) found evidence to support a positive association (n = 1,003 to 9,732), and 2 (50%) found no clear evidence of an association (n = 9,460 to 29,529). All 4 studies that reported an outcome of emotional problems were longitudinal population-based cohorts. Two were Scandinavian (one in Norway found a positive association, one in Denmark found no clear evidence of association), one UK-based (no clear association), and one US-based (positive association).

Total internalising problems

A total of 11 studies investigated the association of maternal PAE with subsequent offspring total internalising problem scores. Of these studies, 5 (45%) found

evidence to support a positive association (*n* = 272 to 607), and 1 (9%) found evidence to support a negative association (*n* = 2,370). The remaining 5 studies (45%) found no clear evidence of an association (*n* = 54 to 37,1525). Of the 5 studies reporting a positive association, 4 studies used a sample with either low SES, offspring with an ADHD diagnosis, or a family history of having an alcohol problem. The one study that reported a negative association used a sample from a Western Australian pregnancy cohort, in which social disadvantage predicted loss to follow up (14 years later). This study therefore represented a sample with higher SES. Of the 5 studies reporting no association, one of these also used participants from the Western Australian cohort. One used a sample of pregnant women with low SES who were offered interventions to reduce alcohol consumption during pregnancy. This study had a low sample size of 54 women and may have been underpowered. One sampled offspring who were prenatally exposed to cocaine. The remaining two studies used participants from the Danish National Birth Cohort.

Total problems

A total of 15 studies investigated the association of maternal PAE with subsequent offspring total problem scores. Of these studies, 8 (53%) found evidence to support a positive association (*n* = 54 to 8,240), and 1 (7%) found evidence to support a negative association (n = 2,370). The remaining 6 studies (40%) found no clear evidence of an association (*n* = 150 to 3,460). Of the 8 studies that reported a positive association, 2 used a sample with low SES, 1 recruited participant based on having ADHD and high alcohol exposure, and one recruited a sample with cocaine exposure, and one study oversampled on mothers with high alcohol consumption. The remaining 3 studies were longitudinal studies of samples from high income countries with sample sizes ranging from 1,003 to 8,240. The one study that found a negative association used participants from a Western Australian pregnancy cohort and were a higher SES sample. Of the 6 studies that did not report an association, 1 also used the Western Australian pregnancy cohort, 4 used UK based longitudinal cohorts, and the remaining study recruited participants at high or low risk of an alcohol problem based on familial history. The one study that reported negative associations between light drinking and offspring total internalising problems and total problem scores, also reported no evidence of an association between heavy drinking and offspring total internalising problems. The sample size of heavy drinking (11 or more drinks per week) within this study (Robinson et al., 2010) was small (*n* = 42), and may therefore have been underpowered to detect a true association.

Conduct disorder

A total of 17 studies investigated the association of maternal prenatal alcohol exposure with subsequent offspring conduct disorder. Of these studies, 9 (53%) found evidence to support a positive association (n = 69 to 8,621), and 1 (6%) found evidence to support a negative association (n = 9,460). The remaining 7 studies (41%) found no evidence of an association (n = 150 to 29,529). Of the studies that reported a positive association, 2 used a sample of children with either social skills deficits or ADHD and heavy alcohol exposure, and 1 used cohort of children being treated at a psychiatric facility. The remaining 6 studies were population-based studies from Western countries, with sample sizes ranging from 69 to 8,621. The one study that reported a negative association used a UK based cohort study, with a large sample size of 9,460. Of the 7 studies that reported no association, 5 of these studies used the same UK based cohort, the Avon Longitudinal Study of Parents and Children (ALSPAC). Of the remaining two studies, one used a sample of the Danish birth-cohort and one recruited participants that were either low or high risk of having an alcohol problem, defined through familial history of alcohol problems.

Two studies used participants from the same cohort (Robinson et al., 2010; Tearne et al., 2015) yet reported contrasting directions of associations with the same measured outcomes (total problems scores; total internalising scores). This may be due to different samples from the same cohort being analysed; both studies controlled for varying covariates resulting in different sample sizes in the fully adjusted models. Each study also measured the original continuous alcohol exposure using separate methods. One study created a binary alcohol exposure measure of ≤ 10 drinks per week compared to > 10 drinks per week (Tearne et al., 2015), and the other created a categorical measure consisting of 5 categories of weekly alcohol consumption (Robinson et al., 2010).

Of the studies that measured total problem scores as the outcome, four studies from the same first author reported using samples from the ALSPAC cohort yet only one study reported a positive association (Sayal et al., 2009), with the remaining three reporting no clear association. This may be due to different exposure measures being used between the studies. One study (Sayal et al., 2009) created a binary measure of binge drinking (≥ 4 units a day) and is therefore measuring drinking patterns and not drinking frequency as 2 other studies were (Sayal et al., 2013; Sayal et al., 2007). The remaining study using the same cohort (Sayal et al., 2014) also measured binge drinking, however they investigated the association with an older age group (11 years) compared to the 2009 study (7 years).

Table 2.1: Studies included in final text stage of systematic review

Author	**Year**	**Country**	**Substance use measure (continuous/ categorical)**	**Gestation period measured**	**Mental health measure (name)**	**Mental health type (anxiety/depression)**	**Method of mental health measure (self-report/ clinical assessment)**	**Summary of results presented in the paper**
(Alvik et al.)	2013	Norway	Categorical: Binge drinking during weeks 0-6 (never; <once a week; ≥ once a week)	0-6 weeks	SDQ	Total problem score, emotional, conduct	Parent report	*Total problems: OR (CI), p* <once a week 1.5 (1.0 to 2.1), 0.05 ≥ once a week 4.1 (1.7 to 9.8), <0.01 *Emotional*: ≥ once a week 3.2 (1.3 to 8.0), <0.05 *Conduct:* ≥ once a week 3.0 (1.3 to 7.2), <0.05
(Bada et al.)	2007	USA	Categorical: yes/no	Doesn't say	CBCL	Internalising, total problem score	Researcher administered	*Internalising: Mean difference (CI), p* 0.61 (-0.01 to 1.24), 0.06 *Total scores:* 0.78 (0.08 to 1.47), .03
(Brown et al.)	1991	USA	Categorical: never drank, stopped drinking, continued to drink after	Doesn't say	CBCL	Internalising, anxious, depressed, total problem score	Teacher & maternal report	*Internalising: F, p* 2.04, ns *Anxious* *<1, ns*

			education intervention					*Depressed* *4.25, 0.02* *Total* 7.16, 0.002
(Chiu, Gau, Tsai, Soong, & Shang)	2009	Taiwan	Categorical. At least once a week, not	Whole pregnancy	CBCL	Anxiety/depression	Parental report	*ns*
(D'Onofrio et al.)	2007	USA	Continuous: Mean number of days exposed to alcohol per week in pregnancy	Whole pregnancy	CBCL	Conduct problems	Mother report	b = 0.06, p < 0.05, SE 0.02
(Day et al.)	2013	USA	Continuous: Average daily volume of alcohol	First trimester	Adult Self-report (continuation of the CBCL)	Internalising, total problem score	Self-report	*Internalising: coefficient, r_2, p* 1.65, 0.004, <0.05 *Total problem score:* 1.9, 0.01, <0.05
(Disney et al.)	2008	USA	Categorical: drank in pregnancy, or not	Whole pregnancy	Structured Clinical Interview for DSM-III-R personality disorders	Conduct disorder	Mother and child report	*Coefficient, p.* 11.59, <.001
(Fryer et al.)	2007	USA	Categorical. Yes or no to alcohol use in pregnancy. Historical records	Whole pregnancy	Kiddie Schedule for Affective Disorders and Schizophrenia for school aged children, present	Depressive disorders, conduct disorder, generalised anxiety disorder	Parent & child report	*Point estimate (CI), p* Depressive disorder: 0.18 (0.08 to 0.31), <.05 Conduct disorder: 0.15 (0.01 to 0.28), p<.05

					and lifetime version and the Computerised diagnostic interview schedule for children, version IV.			GAD: 0.08 (0 to 0.18), p>0.05
(Graham et al.)	2013	USA	Categorical: exposed (>4drinks per occasion at least once), or not	Whole pregnancy	CBCL	Internalising	Parent report	*F* (1,266) = 17.83, p<.001
(Hill, Lowers, Locke-Wellman, & Shen)	2000	USA	Categorical: median split of consumption	1st, 2nd and 3rd trimester	KSADS-Schedule for Affective Disorders and Schizophrenia for school aged children.	Depression, anxiety, conduct disorder, total problem score	Parent, child and psychiatrist report	*OR (CI), p* Depression: 4.48 (1.45 to 13.83), 0.009 Anxiety: 3.27 (1.13 to 9.38), .028 Conduct: 4.42 (1.35 to 14.33), 0.014
(Kelly et al.)	2009	UK	Categorical: Never, light (not more than 1-2 units pw/per occasion), moderate (not more than 3-6units pw, 3-5 units per occasion), heavy/binge (≥7 units pw or >6 units per occasion)	Asked retrospectively after birth (child 9 months)	SDQ	Conduct, emotional, total problem score	Parent report	*Boys: OR (CI)* *Total*: Light 0.77 (0.56 to 1.07) Moderate 0.65 (0.35 to 1.23) Binge 1.76 (0.83 to 3.73) *CD*: Light 0.59 (0.44 to 0.81) Moderate 0.68 (0.39 to 1.21) Binge 0.53 (0.22 to 1.27) *Emotional*: Light 0.85 (0.60 to 1.21), Moderate 0.81 (0.40 to 1.64) Binge 2.15 (1.09 to 4.25)

								Girls: *Total*: Light 0.70 (0.43 to 1.14) Moderate 1.18 (0.63 to 2.19) Binge 0.83 (0.30 to 2.28) *CD*: Light 0.72 (0.52 to 1.00) Moderate 1.60 (0.92 to 2.78) Binge 1.18 (0.49–2.83) *Emotional*: Light 0.95 (0.65 to 1.38) Moderate 0.90 (0.45 to 1.79) Binge 1.62 (0.72 to 3.68)
(Kendler et al.)	2013	USA	Doesn't state	18 and 32 weeks gestation	SDQ	Conduct disorder & emotional problems	Maternal report	*Beta, SE, p* *Conduct:* *0.052, 0.014, <0.001* *Emotional: 0.038, 0.014, <0.01*
(Knopik et al.)	2009	USA	Categorical: 1-10 days, 11-35 days, >35 days, some heavy use (at least 5-6 drinks on days and at least once a month), frequent heavy use (5+ drinks on 2-3 days pm)	Whole pregnancy	DICA for telephone administration	Conduct disorder	Self-report	*Beta, SE, p* *1-10 days use: -.001, .024* *11-35 days use: -.077, .058* *>35 days use: .095, .132* *Some heavy use: -.053, .093* *Frequent heavy use: .388, .158, p<.01*
(Larkby et al.)	2011	USA	Categorical: none (adv=0), light (adv≤0.4), moderate (>0.4, ≤0.89), heavy (>0.89)	1st trimester	DIS-IV	Conduct disorder	Structured interview	*First trimester:* p = 0.002

(Murray et al.)	2016	UK	Categorical: Moderate drinking (>0-6 units pw at any time in pregnancy), and not drinking >6 units pw on a single occasion at any time in pregnancy	18 and 32 weeks gestation	SDQ	Conduct disorder	Self-report	$p = 0.633$
(Niclasen, Nybo Andersen, Teasdale, & Strandberg-Larsen)	2014	Denmark	Cumulative intake of alcohol	Whole pregnancy	SDQ	Internalising	Parent report	Boys OR (CI) 0: 1.03 (0.99 to 1.08) >0-5: 1.02 (0.98 to 1.06) 1.02 (0.99 to 1.06) >15-45: ref >45-90: 0.99 (0.96 to 1.03) >90: 0.92 (0.88 to 0.97)
(Niclasen, Andersen, Strandberg-Larsen, & Teasdale)	2014	Denmark	Categorical: binge drinking (5+) drinks in early, or late pregnancy, and never	16 and 30 weeks gestation	SDQ	Conduct, emotional, internalising	Parent report	*Internalising: relative change in mean (CI)* Early binge 1.00 (0.98 to 1.03) Late binge 1.05(0.89 to 1.24) *Conduct: OR (CI)* Early binge 1.01 (0.91 to 1.11) Late binge 0.81 (0.49 to 1.43) *Emotional: OR (CI)* Early binge 0.93 (0.84 to 1.03) Late binge 0.86 (0.51 to 1.57)

(O'Connor & C. Kasari)	2000	USA	Categorical: Abstinent-light (0-2 drinks per occasion), moderate-heavy (3 or more drinks per occasion)	Whole pregnancy	Pictorial Depression Scale	Depression	Self-report	*t (39) = 2.02, p < 0.05*
(O'Connor & Paley)	2006	USA	Continuous: Maximum drinks per drinking occasion	Whole pregnancy	Pictorial Depression Scale	Depression	Self-report	*r* =0.35, *p*<0.05
(O'Connor)	2001	USA	Continuous: Maximum drinks per occasion	Whole pregnancy	Pictorial Depression Scale	Depression	Self-report	*r* = .43, *p*<.01
(O'Leary & Bower)	2009	Australia	Categorical: abstinent, low (over a week <7 drinks AND on any day no more than 1-2 standard drinks), moderate (10g of alcohol per occasion) daily, heavy (5 or more per occasion)	1st trimester	CBCL	Anxiety/depression, internalising, total problem score	Parent report	*OR (CI)* *Anxiety/depression*: Low 1.06 (0.59 to 1.88) Moderate 2.24 (1.16 to 4.34) Heavy 2.82 (1.07 to 7.43) *Internalising:* Low 1.04 (0.73 to1.49) Moderate 1.14 (0.67 to 1.94) Heavy 2.65 (1.36 to 5.14) *Total:* Low 0.97 (0.69 to 1.37) Moderate 1.17 (0.74 to 1.84) Heavy 1.62 (0.85 to 3.11)

(O'Leary et al.)	2010	Australia	Categorical: abstinent, <10g alcohol per occasion, 10g alcohol daily & binge drinking <weekly, binge (60-70g per week).	1st trimester	CBCL	Anxiety/depression	Parent report	*First trimester OR (CI)* Low 1.06 (0.59 to 1.88) Moderate 2.24 (1.16 to 4.34) Heavy 2.82 (1.07 to 7.43)
(Robinson et al.)	2010	Australia	Categorical: abstinent, occasional (up to 1 drink pw), light (2-6 drinks pw), moderate (7-10 drinks pw), heavy (≥11 drinks pw)	18 weeks gestation	CBCL	Total problem score, internalising	Parent report	*OR (CI), p* *Internalising* *18 weeks* Occasional: 0.85 (0.67 to 1.07) 0.164 Light: 0.57 (0.42 to 0.76), <0.001 Moderate: 0.31 (0.14 to 0.69), 0.004 Heavy: 0.76 (0.33 to 1.76), 0.519 *Total* Occasional: 0.82 (0.63 to 1.06), 0.133 Light: 0.63 (0.46 to 0.86), 0.003 Moderate: 0.43 (0.21 to 0.88), 0.020 Heavy: 0.68 (0.31 to 1.47), 0.323
(Sayal) *et al.*	2007	UK	Categorical: <1 glass pw, ≥1 glass pw	18 weeks gestation	SDQ	Conduct disorder	Parent and teacher report	*OR (CI)* *82 months* <1 glass: 1.18 (0.99 to 1.40)

								≤1 glass: 1.20 (0.95 to 1.52)
(Sayal et al.)	2009	UK	Categorical: ≥ 4 drinks in a day on any one occasion, < 4 drinks in a day on any occasion	18 and 32 weeks gestation	SDQ	Conduct problems, total problem score	Parent report	*Conduct. Coefficient (CI), p* *0.12 (0.02 to 0.22), 0.020* *Total* *0.36 (0.04 to 0.68), 0.026*
(Sayal et al.)	2013	UK	Categorical: <1 glass pw, ≥1 glass pw	18 weeks gestation	SDQ	Conduct disorder, total problem score	Parent report	*Conduct. Coefficient (CI), p* <1 glass: 0.06 (-0.02 to 0.14), 0.151 ≥1 glass: 0.04 (-0.07 to 0.15), 0.462 *Total* <1 glass: 0.13 (-0.14 to 0.40), 0.347 ≥1 glass: 0.04 (-0.33 to 0.42), 0.825
(Sayal et al.)	2014	UK	Categorical: Binge drinking. Consumed <4 drinks (includes non-drinkers), and ≥ 4drinks, at any time in pregnancy	18 and 32 weeks gestation	SDQ	Conduct disorder, total problem score	Parent and teacher report.	*Conduct. Coefficient (CI), p* 0.05 (-0.06 to 0.15), 0.406 *Total* 0.30 (-0.07 to 0.67), 0.109
(Silva, Houghton, Hagemann, & Bower)	2015	Australia	Categorical: yes/no	Whole pregnancy	Doesn't say	Depression, anxiety	Parent report	*OR (CI)* Depression: 1.08 (0.55 to 2.12) Anxiety: 1.14 (0.66 to 1.97)

(Sood et al.)	2001	USA	Categorical: Average absolute alcohol pd across pregnancy. Low (>0-<0.3fl oz/pd), moderate/heavy (≥0.3fl oz)	Whole pregnancy	CBCL	Internalising and total score	Parent report	R_2, β, p Internalising: 0.274, 0.096, 0.020 Total score: 0.308, 0.114, .005
(Staroselsky et al.)	2009	Canada	Documented history of alcohol exposure in utero, or not	Not stated	CBCL	Anxiety and depression	Not stated	*ns*
(Tearne et al.)	2015	Australia	Categorical: ≤10 drinks pw, 11> drinks pw	18 weeks gestation	CBCL	Total problem score, internalising	Parent report	*ns*
(Walthall et al.)	2008	USA	Categorical: Exposure levels of <1 standard drink in gestation, drank in pregnancy	Whole pregnancy	National Institute for Mental Health Computerized Diagnostic Interview Schedule for Children-Fourth Edition	Disorders of: Separation anxiety, generalised anxiety, Conduct Disorder, major depressive disorder	Parent or caregiver report	*F*, *p*, β, *B(SE)*, r_2*(adjusted* r_2*)* *Separation anxiety disorder*: 9.82, <.001, 0.24, 13.35(5.62), 0.13(.12) *Generalised anxiety disorder*: 6.27, 0.001, 0.17, 8.35(4.18), 0.09(.08) *CD:* 9.69, <.001, 0.24, 7.62(3.19), 0.24(.21) *Major depressive disorder:* 6.27, <.001, 0.15, 6.98(4.89), 0.17(.14)

(Ware et al.)	2013	USA	Categorical: Control: no more than 1 drink pw on average, and never more than 2 drinks on any single occasion, >4 drinks at least once in pregnancy	Whole pregnancy	Computerised Diagnostic Interview Schedule for Children-IV and CBCL	Anxiety/depression, withdrawn/depression, Internalising, total problem score	Parent completed	*Anxious depressed:* F=9.70, p=.002 *Withdrawn/depressed:* F=9.25, p=.003 *Internalising*: F=22.32, p<0.001 *Total problems:* F= 46.61, p<0.001

PAE: prenatal alcohol exposure, pm: per month, pd: per day, *ns*: reported as "non-significant"

2.4 Discussion

The aim of this systematic review was to investigate the association between maternal alcohol use during pregnancy and offspring mental health, by appraising the current literature and describing the findings. In general, available evidence suggests that alcohol use during pregnancy is associated with increased risk of mental health problems in the offspring, specifically anxiety/depression, total problems and conduct disorder. Of the five extracted outcome types, three types of mental health (anxiety/depression, total problems and conduct disorder) showed a majority reporting a positive association. An equal number of studies reported both a positive association and no clear evidence to support an association, between maternal alcohol use in pregnancy and emotional problems, as well as total internalising scores. Only two studies showed that increased alcohol exposure during pregnancy was associated with *increased* positive mental health in offspring. In one of these studies (Kelly et al., 2009), the authors suggest that the J-shaped curve shown in their results may not actually be due to light drinking in pregnancy causing a reduction in offspring mental health, but instead due to residual confounding.

There are limitations that should be considered when interpreting these results. First, as all the included studies are observational, their findings may still be influenced by the well described problems of residual confounding. Both the amount, and type of confounders that were adjusted for also varied greatly between studies, making comparisons across studies to assess any consistent effects difficult when assessing confounding influences. Of note is the different approach to adjustment for maternal drug use during pregnancy across studies. Bada and colleagues (Bada et al., 2007) assessed children prenatally exposed to cocaine and adjusted for alternative illicit drug use such as opiates and marijuana; however, few other studies included prenatal drug use, and those that did mainly adjusted for marijuana use only. Few studies adjusted for maternal mental health, which has been shown to influence offspring mental health (McQuire et al., 2019; Pearson et al., 2013) and therefore could be inflating associations shown. Studies included also did not adjust for parental postnatal alcohol use which could potentially have an influence on their own psychopathology, and subsequently their child's.

Second, varying methods were used for exposure and outcome measurements between studies. Of the 33 studies included in this review, all but four used a different measure of prenatal alcohol use, with varying definitions of "low" or "moderate" alcohol exposure. As there is no universally accepted definition of low, moderate or heavy alcohol use in pregnancy (Sood et al., 2001), comparisons between studies is difficult. This substantial heterogeneity between studies meant that a meta-analysis was inappropriate for this review. Differences were also shown across studies for the method

of report for prenatal alcohol exposure (e.g., self-report, medical report), and at what timepoint alcohol use was recorded (e.g., early or late pregnancy, after birth). As a result of this, the stage of pregnancy maternal alcohol use may have the greatest effect on offspring mental health cannot be estimated. If PAE was measured retrospectively or prospectively was also varied between studies. Such measurements could have influenced the report of alcohol use as prospective studies are more reliable and less prone to recall bias.

Third, there was substantial variation in the length of follow up times (3 to 26 years). The study that measured the oldest age group within this review (Day et al., 2013), found intrauterine alcohol exposure was associated with total problem scores in offspring at a mean age of 22, which suggested the associations shown at earlier ages may be present in early adulthood. However, replication using older age groups is required to confirm this, as all other studies within this review except for one (Larkby et al., 2011) investigated a sample of offspring aged 16 or younger. Fourth, sample sizes ranged from 41 to 37,315 offspring, and some of the smaller studies may have been underpowered. Different diagnostic tests with varying cut-offs for determining clinical thresholds were used to assess offspring mental health, measured by self-report, parental/carer report, or teacher report. Although some studies within this review used the same measures, they did not always report every subscale within each test. For example, the CBCL measures a variety of subscales, but often studies only utilised the total summed score. This made it further difficult to assess which subscale of internalising disorders may be contributing to the total score.

Fourth, non-English language publications were excluded from this review. The exclusion of such studies could have led to bias. Previous literature comparing the findings from English language and German language journals, reported randomised control trials more likely be published in an English language journal if they found a p value of $p<0.05$ (Egger et al., 1997). In the current review only one study was excluded for being a non-English publication and the likelihood of overall bias is small. However, future systematic reviews should aim to include non-English studies also to reduce the possibility of publication bias. An assessment for risk of bias was not conducted within this review, which could mean the findings within this review may be over or underestimating the reported direction of results. There are many different reporting tools for assessing risk of bias within systematic reviews. However, there are limitations to the existing assessment tools such as their given guidance in making assessments, their scope and measurements used relative weight that is given within each assessment tool in unclear (Page, McKenzie, & Higgins, 2018). Such reporting tools may be further useful in exploring heterogeneity within meta-analyses. A lack of quality assessment used

within this review could mean that we should be less certain that the findings reported within included studies are the true representation of the outcome. Future studies may therefore benefit from using a formal assessment of both methodology and reporting quality within studies.

This review describes and summarises the findings for published literature investigating maternal prenatal alcohol exposure and offspring mental health. It also details the limitations in being able to create a synthesis of results due to the marked differences in exposure, and outcome measurement across studies, including types of measures/subscales used, method of report and length of follow up. I propose that future studies within this area should aim to use a detailed measure of alcohol frequency across trimesters instead of simply a binary measure of the presence/absence of alcohol use at any point during gestation. This would allow the reader to infer the amount of alcohol and timing of exposure which may be associated with offspring outcomes. This may also enable a synthesis of results in a meaningful meta-analysis. The inclusion of similar outcome measurements to previous research would also be advantageous, however due to the limitations in available measurements within studies, it is instead suggested that future studies describe the findings for each subscale within internalising measures, as opposed to merely stating 'total' scores. The current review also highlights the disparity in which age internalising outcomes have been measured, with many focusing on younger age groups. Within studies this may be due to the younger age of available participants, however with the length of follow up for many cohort studies now increasing, it is suggested that future studies also focus on older age groups to investigate if any associations shown at for earlier ages continue into adulthood and replicate those that have suggested it may (Day et al., 2013).

Only English language studies were included in this review, which may have led to the omission of some studies. However, it has been reported that little evidence of bias is introduced from the exclusion of non-English studies (Morrison et al., 2012). Studies were also only included if they were published. By not including unpublished studies this means that low quality studies were unlikely to have been included, however this could mean that publication bias may have affected our results as positive findings are often more likely to be published. If non-published studies were included, there may have been more null results.

Two of the outcome categories included an externalising component (conduct disorder and total problem scores). Total problem scores were often calculated from the individual mental health subscales, and which subscales were included in this total varied across measures and studies. This means it is difficult to summarise how much of the total problem score is attributed purely to internalising or externalising disorders.

2.4.1 Future studies

The longitudinal studies which were included within this review can identify associations but do not provide evidence of causality on their own. Future studies could therefore utilise methods that allow stronger causal inference, such as negative control analyses and Mendelian Randomization (MR) if possible. However, this is not always the case. For example, genetic variants currently identified for alcohol use suffer from weak instrument bias and can have reduced power to detect a true effect especially when used in a study with a small sample size. MR is therefore not often a suitable approach in investigating the effect of prenatal alcohol exposure on offspring mental health. Negative control analyses can instead be used to show if an association is still observed by a different exposure that is likely to have a similar confounding structure to the original exposure of interest, but no biological link (Gage, Munafò, & Davey Smith, 2016). If an association is also found within the negative control analyses, this is likely to be due to confounding and not due to the original exposure of interest (Davey Smith, 2008). When investigating the potential causal influence of maternal alcohol use in pregnancy on offspring outcomes, paternal alcohol use during pregnancy can be used as a negative control, as paternal alcohol use during pregnancy can have no direct biological effect on the developing fetus. Triangulation of multiple approaches (Lawlor, Tilling, & Davey Smith, 2016) would allow researchers to investigate the causal effects of maternal alcohol use during pregnancy.

In summary, this review helps to address a gap in the literature by systematically reviewing published research on intrauterine alcohol exposure and offspring mental health for all ages above 3. I found evidence of a positive association between maternal prenatal alcohol use and offspring mental health problems, specifically anxiety and depression, conduct disorder and total problem scores. As the alcohol exposures between studies were all measured using different scales, it is difficult to discern what level of intrauterine alcohol exposure is related to each mental health outcome. As this review excluded studies that measured FAS outcomes specifically, the novel design means I am more certain that the results obtained are for lower levels of alcohol use. However, as this review sought to evaluate the subclinical effects of alcohol use by excluding predefined groups with FAS, the current review still cannot be certain that the included studies are not still capturing offspring with undiagnosed FASD. This is due to a lack of formal categorisation of how much intrauterine alcohol exposure is required to cause FAS/FASD and be clinically dangerous to the developing fetus. The exact relationship between

FASD and ADHD remains unclear, however, ADHD is the most commonly reported mental health diagnosis for children exposed to maternal alcohol use during pregnancy (Fryer et al., 2007). Some studies included within this review recruited a sample of offspring with an ADHD diagnosis. As ADHD has been suggested to be a clinical subtype of FASD (Peadon & Elliott, 2010), this may mean that the inclusion of samples with ADHD diagnosis may actually have been capturing offspring with FASD.

Despite the high amount of heterogeneity across studies, and differences in study design I still evidenced a predictable positive association between low levels of alcohol exposure and offspring mental health problems. These findings give support for future work to further investigate children with low levels of intrauterine alcohol exposure, as well as the need to focus on causal inference and rule out alternative explanations behind these findings.

2.5 Chapter Summary

In this chapter I aimed to systematically review the previous literature on PAE and offspring mental health, specifically internalising disorders and conduct disorder. My findings suggest that maternal alcohol use during pregnancy is associated with increased risk of mental health problems in offspring at various ages, specifically for mental health outcomes of anxiety/depression, total problems and conduct disorder. This chapter highlights the disparity between studies for how PAE is defined, meaning comparisons across studies is challenging. It also highlights how that due to the availability of suitable data from different cohorts, most previous studies utilise child or pre-adolescence age groups when measuring mental health outcomes in offspring. This means it is therefore harder to say with certainty if any effects found are likely to be present in early adulthood also. In the next chapter I will build on the existing literature described in Chapter 2 by using a longitudinal cohort study, the Avon Longitudinal Study of Parents and Children (ALSPAC). I have included measures of alcohol frequency, amount and pattern within two different stages of pregnancy to investigate PAE. I also used partner alcohol consumption within a negative control design to aid causal interpretation.

Chapter 3 Association of intrauterine alcohol exposure and offspring depression: A negative control analysis of maternal and partner consumption

A version of this chapter is currently in press at the *Journal of Alcoholism: Clinical and Experimental Research*. It is also currently available online as Easey, K. E., Timpson, N. J., Munafò, M. R. (2019). Association of intrauterine alcohol exposure and offspring depression: A negative control analysis of maternal and partner consumption. bioRxiv, doi: http://dx.doi.org/10.1101/307462.

As discussed in Chapter Two the findings from the systematic review demonstrate a lack of studies investigating PAE and offspring internalising outcomes within older age groups, meaning it is challenging to ascertain if any associations shown for the earlier age groups persist into adulthood. The previous literature had also yet to incorporate methods to further aid causal inference such as a negative control design.

In this chapter I investigate associations between both maternal and partner prenatal alcohol use and offspring depression employing a negative control design using the ALSPAC cohort. PAE included measurements of both frequency, amount, and pattern of parental alcohol use by measuring how often and how much mothers and partners drank alcohol, and how often they binge drank alcohol.

3.1 Introduction

Alcohol consumption during pregnancy is common. PAE amounts vary between studies, with up to 80% of expectant mothers in the UK, Australia and New Zealand having reporting PAE (O'Keeffe et al., 2015). However, a more recent systematic review and meta-analysis which investigated the prevalence of alcohol use in pregnancy across varying countries, reported up to 41.3% of expectant mothers in the UK consuming alcohol during pregnancy, and a global prevalence of 9.8% (Popova, Lange, Probst, Gmel, et al., 2017). This high percentage of women reporting alcohol use may be in part due to previous guidelines, which suggested that low levels of consumption are safe for the developing fetus. Until recently in the UK, for example, guidelines advised pregnant women to abstain from alcohol in the first three months of pregnancy; however, as discussed in Chapter One these guidelines also stated that there is no evidence that a low level of alcohol use of 1-2 units (2 units being a 175ml glass of 12% ABV wine), no more than once or twice a week is linked to harm to the unborn child (NICE, 2008). Guidelines

for alcohol use during pregnancy have only recently been updated to advise that women should abstain from alcohol consumption during their entire pregnancy. This change is based on a precautionary principle in the absence of robust evidence (DOH, 2016).

It is well established that heavy alcohol use in pregnancy can cause fetal alcohol syndrome (Jones & Smith, 1973; Mukherjee et al., 2006), resulting in physical and cognitive impairments (Coles, Platzman, Lynch, & Freides, 2002; Gibbard, Wass, & Clarke, 2003; Guerri, Bazinet, & Riley, 2009). However, even at levels of alcohol consumption below that required for fetal alcohol syndrome, exposure to alcohol during gestation has been shown to be associated with detrimental outcomes in offspring, such as being small for gestational age (Mamluk et al., 2017), birth complications such as pre-eclampsia and placental abruption (Salihu et al., 2011), as well as behavioural outcomes such as increased risk of externalising disorders (Sayal et al., 2014) and internalising disorders (Sood et al., 2001; Walthall et al., 2008). However, a recent review meta analysed the literature conducted within such areas and found no consistent evidence (Mamluk et al., 2017) for the influence of light alcohol exposure. There is no known dose threshold for causing FAS, with other important contributing factors influencing FAS presentation such as timing of exposure, nutritional status and individual vulnerability (Maier & West, 2001).

However, much research in this area has been conducted on offspring at an early age, with less research in older age groups to establish whether these associations persist into adulthood. One of the few studies to have used an older offspring age group suggested that the detrimental outcomes shown for gestational exposure to alcohol are likely to be present in early adulthood also as they were still evident at age 22 (Day, A Helsel, Sonon, & Goldschmidt, 2013), although replication of this finding is required. Low levels of intrauterine alcohol exposure have also been shown to be protective against offspring internalising and externalising problems in some studies (Kelly et al., 2009; Robinson et al., 2010). Studies utilising more robust methods to aid in causal inference such as Mendelian Randomization (MR) and sibling control designs have suggested that residual confounding may exist and be accounting for such observed associations (D'Onofrio et al., 2007; Murray et al., 2016).

As also shown in chapter Two, frequency, pattern and timing have also been shown to be important when investigating maternal alcohol use in pregnancy, as opposed to just the presence or absence of consuming alcohol (O'Leary et al., 2010). Day and colleagues reported a dose-response association for alcohol use during pregnancy across all three trimesters with increased offspring mental health problems (Day et al., 2013). However, the evidence is mixed for specific associations during different trimesters. Niclasen and colleagues reported evidence that binge drinking at both 16 and 30 weeks

gestation is associated with conduct disorder (Niclasen, Andersen, et al., 2014a). On the other hand, O'Leary and colleagues did not find evidence of an association with internalising disorders when their analyses were restricted to the third trimester (O'Leary et al., 2009). Observational studies such as these can identify associations. However, the well described problems of bias, reverse causation and confounding, are likely responsible for conflicting evidence on the effects of intrauterine alcohol exposure, and causal inference is difficult. Selection bias can occur within longitudinal studies if higher rates of attrition are shown for certain demographic features of a cohort. For example, if greater or fewer families from a higher socio-economic background are retained over a long follow up, this can ultimately reduce the internal and external validity of any findings (Hill, Rosenman, Tennekoon, & Mandal, 2013).

Methods which aid in inferring causal inference are increasingly used in observation epidemiology. These methods, such as MR, sibling control designs and negative controls help to lessen the problems of bias, reverse causation and confounding. MR is a method used to generate estimates of causal association using genetic variants that are robustly known to be associated with exposures as a proxy for modifiable behaviours (Davey Smith, 2008) and can go some way to protect against the limitations of observational epidemiology (Davey Smith & Ebrahim, 2004). However, genetic variants identified for alcohol use to date have small effect sizes and might suffer from weak instrument bias, therefore reducing power to detect a true effect. Sibling designs where offspring were exposed to discordant health behaviours, can be used to indicate bias or confirm results in findings from population studies (Keyes, Davey Smith, & Susser, 2013). However, it can be challenging to find siblings discordant for certain health behaviours during pregnancy. Negative control analyses are another method to assess whether associations are due to confounding, or likely to be causal. This is done by using exposures or outcomes with similar confounding structures but no plausible biological link (Gage et al., 2016). If an association is also shown in the negative control analyses, it is more likely to be due to confounding and not the original exposure of interest (Davey Smith, 2008). Comparison of parental exposures on offspring outcomes can be used to test intrauterine effects. For example, both maternal and paternal drinking are likely to be influenced by similar confounding, and therefore if an association with offspring outcomes is observed it will be more likely due to maternal mechanisms. As maternal mental health is likely to confound any association shown for PAE and offspring mental health (Kingston & Tough, 2014; Leis, Heron, Stuart, & Mendelson, 2014), the use of a negative control analyses within this chapter is particularly advantageous as partner mental health would also likely be associated with offspring depression and their own alcohol use.

Negative control analyses have been previously used to investigate the effects of smoking during pregnancy on offspring mental health (A. E. Taylor et al., 2017). There are currently few studies that have used using negative control analyses to investigate parental alcohol use during pregnancy and these have mainly focused on offspring externalising disorders (Eilertsen et al., 2017) or general cognitive ability (Alati et al., 2008). I therefore sought to investigate associations between both the frequency and pattern of maternal drinking in pregnancy (at multiple available timepoints during gestation) and offspring depression, using data from a population based longitudinal study. I also investigated whether any associations may reflect a causal effect, using negative control analyses of partner drinking in pregnancy on offspring depression, as both these exposures are likely to be influenced by similar confounding.

3.2 Methods

3.2.1 Sample

ALSPAC is an ongoing population-based study, which recruited pregnant women residing in Avon, UK with expected dates of delivery between 1st April 1991 to 31st December 1992. The core sample consisted of 14,541 pregnant women, of which 14,062 were live births and alive at 1 year of age. Participants have been regularly followed up through clinic visits and questionnaires. Detailed information about ALSPAC is available on the study website which includes a fully searchable data-dictionary of available data (http://www.bris.ac.uk/alspac/researchers/data-access/data-dictionary). For further details on the cohort profile, representativeness, and phases of recruitment, see articles by Boyd and colleagues, and Fraser and colleagues, (Boyd et al., 2013; Fraser et al., 2013). Ethics approval for the study was obtained from the ALSPAC Ethics and Law Committee and the Local Research Ethics Committees.

3.2.2 Measures

Exposures. Alcohol consumption during pregnancy was measured by: 1) Frequency of drinking: mothers and partners were asked separately the frequency and amount of alcohol consumed (within the past 3 months) at 18 weeks gestation. Response categories were never, <1 glass per week, 1+ glass per week, 1-2 glasses a day, 3-9 glasses a day and ≥10 glasses a day. For the analyses, the last two categories were combined to 3+ glasses a day. 2) Pattern of drinking (binge drinking): mothers and partners were asked the number of days they had drank the equivalent of 2 pints of beer, 2 glasses of wine, or 4 measures of spirits or more. The definition of binge drinking used here does not necessarily align with certain definitions of binge drinking/binging (Stahre,

Roeber, Kanny, Brewer, & Zhang, 2014). A definition of binge drinking is however given here to distinguish it from lower levels of alcohol consumption which does not exceed one drink per day. This definition of heavy/binge drinking has been previously used and reported in multiple studies (Alati et al., 2013; Mahedy et al., 2017; Sayal et al., 2009). Mothers were asked at both 18 weeks (how many times within the last 3 months) and 32 weeks (how many times within the last month) gestation; partners were asked at 18 weeks gestation. Response categories were 0, 1-2 days, 3-4 days, 5-10 days, >10 days and every day. For my analyses, the last two categories were combined to >10 days.

Outcomes. Offspring depression was measured using the Clinical Interview Schedule-Revised (CIS-R) through self-report, which is validated in reliability studies (Lewis, Pelosi, Araya, & Dunn, 1992). The CIS-R is a computerised interview consisting of questions that are scored between 0-5, with 5 depicting greater levels of depressive symptoms. These scores were then totalled, and scores greater than 12 indicated a clinical level of depression according to ICD-10 criteria and were used as a binary measure of depression. The self-completion computerised version of the CIS-R has been shown to have good validity with interviewer administered versions of the CIS-R, showing means of 4.35 and 4.43 for difference in mean scores, and no differences for symptom scores for 12 of the 14 symptom scores (Head et al., 2013). Subsequent sensitivity analyses were conducted using the CIS-R measure at age 24, to investigate whether any associations observed are also present at a later age.

Confounders and sensitivity analysis. Potential confounding factors associated with both alcohol consumption and offspring psychiatric disorder were included in the analysis. These were factors that I wanted to remove from any analyses within an adjusted model to investigate what associations/if any remained after adjustment. These consisted of socio-economic variables, social factors, as well as maternal health behaviours added within separate adjusted models (see section 3.2.3). Included confounders were mother's socioeconomic position (professional/managerial or other) measured during pregnancy, income (divided into quintiles) measured at age 3 and 4 years, home ownership (mortgage/non-mortgage) measured at 8 weeks gestation, marital status (married or not) measured at 8 weeks gestation, maternal education (university degree/<university degree), sex, parity (first born, 2+ born), maternal tobacco (yes/no) and illicit drug use (yes/no) in months 1-3 of pregnancy, and maternal depression at 18 weeks gestation (scores >12 highly associated with a diagnosis of depression) measured by the Edinburgh Postnatal Depression Scale (EPDS) (Cox, Chapman, Murray, & Jones, 1996).

3.2.3 Statistical analyses

I used logistic regression to investigate associations between maternal and partner alcohol frequency (18 weeks gestation), binge drinking (18 and 32 weeks gestation) and a diagnosis of depression (CIS-R) in offspring at 18 years of age. Comparisons were made between the never drank in pregnancy controls in each alcohol exposure and each alcohol frequency/pattern group. Analyses were conducted using Stata version 14.2.

The impact of confounders on these associations was explored by comparing unadjusted estimates, to those adjusted for socioeconomic variables (adjusted model 1) and those further adjusted for maternal behaviours during pregnancy (e.g., other drug use during pregnancy/maternal depression) (adjusted model 2), and for partner alcohol use (frequency or pattern, dependant on exposure) during pregnancy variables (18 weeks gestation only) (adjusted model 3). By increasing the number of items adjusted for the sample size decreased, as individuals with missing data are excluded from analysis. Therefore, as a sensitivity analysis, all analyses were conducted using the full sample and then repeated only on participants with complete data.

Multiple imputation by chained equation (MICE) in Stata (Royston & White, 2011) was also used to generate a maximum dataset comprising of 100 imputed datasets, each with 10 cycles. Generation of more than one imputation model allowed for the uncertainty in predicting missing data, by adding variability to the imputed values in each dataset, which are then averaged together. The variability in results between each dataset reflect the uncertainty associated with the missing values, and using Rubin's rules standard errors are calculated which account for the variability in these results (Sterne et al., 2009). By averaging the distribution of the missing data from the observed data, valid assumptions can be made which account for variability. This method assumes any systematic differences between the missing and observed values can be explained by differences in observed data and are Missing at Random (Sterne et al., 2009). Multiple auxiliary variables available from the ALSPAC cohort were used to assist in the imputation. These included the predictive factors used in the main analysis (e.g., socioeconomic position), as well as other measures related to the outcomes (e.g., EPDS), and earlier offspring depressive measures such Mood and Feelings Questionnaire (MFQ) (Angold et al., 1995).

3.3 Results

Overall, within the full sample 16% of mothers (2,088 of 13,195) reported drinking at least one alcoholic drink per week in the first three months of pregnancy. At 18 weeks gestation 17% of mothers (2,234 of 13,149) reported binge drinking for at least

1-2 days within the past month. For mothers who provided information on alcohol frequency or pattern of drinking, 4,191 and 4,169 offspring respectively provided information for CIS-R diagnosis of depression at age 18 (see Figure 3.1 for included participants). Mother and offspring characteristics for full sample analysis are presented in Tables 3.1-3.4. All further presented results are for imputed analyses unless stated otherwise.

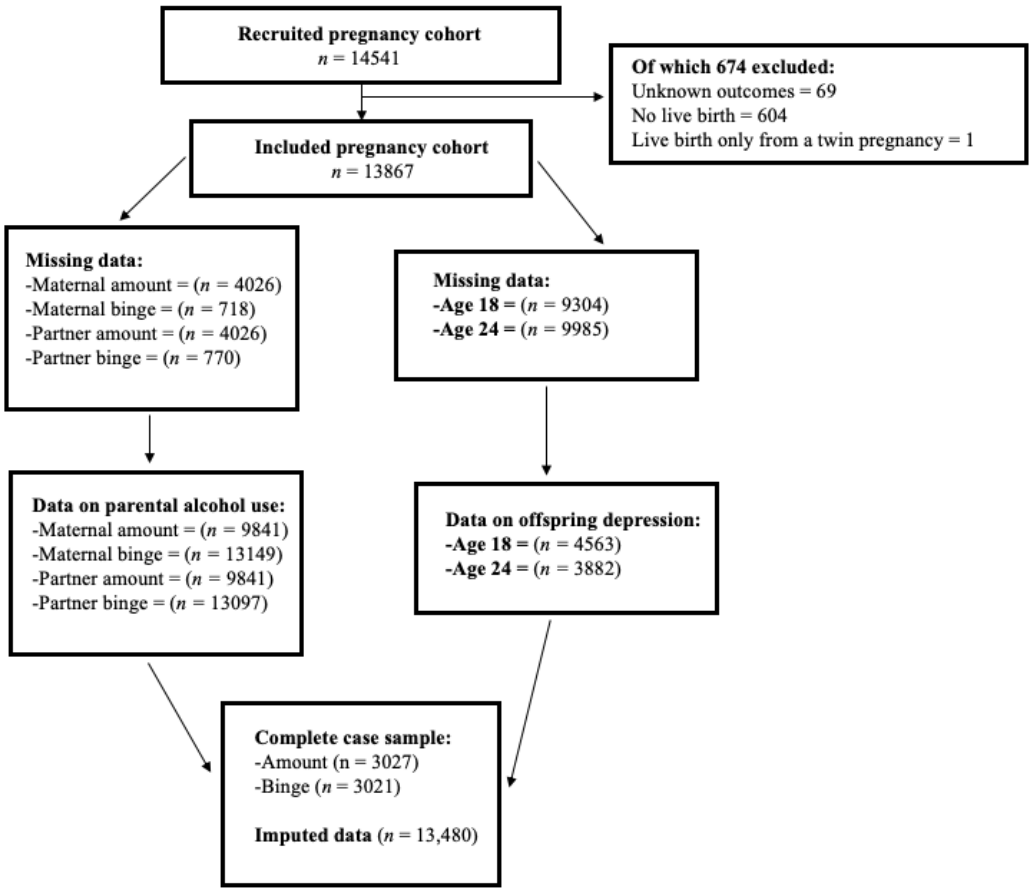

Figure 3.1: Flow chart of the mothers, partners and offspring included in the final sample

Table 3.1: Socioeconomic and offspring factors, by maternal pattern of drinking (number of times 4+ units of alcohol in past month at 18 weeks gestation)

	Drinking patterns (maternal)					
	None	1-2 days	3-4 days	5-10 days	>10 days	Total
Socioeconomic position (*n* = 9876)						
i-ii	3212	276	116	52	59	3715
(%)	(87)	(7)	(3)	(1)	(2)	
iii-v	5071	595	247	130	118	6161
(%)	(82)	(10)	(4)	(2)	(2)	
Home ownership (*n* = 12647)						
Home owner	7975	754	313	158	167	9367
(%)	(85)	(8)	(3)	(2)	(2)	
Non-home owner	2547	378	146	99	110	3280
(%)	(78)	(12)	(4)	(3)	(3)	
Marital status (*n* = 12697)						
Married	8213	753	296	164	163	9589
(%)	(86)	(8)	(3)	(2)	(1)	
Not married	2363	379	161	92	113	3108
(%)	(76)	(12)	(5)	(3)	(4)	
Maternal education (*n* = 12043)						
University degree	1440	84	23	19	19	1585
(%)	(91)	(5)	(2)	(1)	(1)	
No university degree	8603	980	415	221	239	10458
(%)	(82)	(10)	(4)	(2)	(2)	
Offspring sex (*n* = 13075)						
Male	5612	629	234	137	153	6765
(%)	(83)	(9)	(4)	(2)	(2)	
Female	5248	553	245	129	135	6310
(%)	(83)	(9)	94)	(2)	(2)	
Parity (*n* = 12923)						

First born	4922	474	178	109	103	5786
(%)	(85)	(8)	(3)	(2)	(2)	
2nd+ born	5812	694	291	158	182	7137
(%)	(81)	(10)	(4)	(2)	(3)	
Smoked during pregnancy (*n* = 13149)						
No	8496	740	267	149	166	9818
(%)	(87)	(8)	(3)	(2)	(2)	
Yes	2419	452	215	118	127	3331
(%)	(73)	(13)	(6)	(4)	(4)	
Drug use during pregnancy (*n* = 12982)						
No	10748	1168	464	255	284	12919
(%)	(83)	(9)	(4)	(2)	(2)	
Yes	33	7	11	7	5	63
(%)	(52)	(11)	(17)	(11)	(8)	
Depression 18 weeks gestation (*n* = 12982)						
No	8744	894	334	188	198	12919
(%)	(84)	(9)	(3)	(2)	(2)	
Yes	1260	195	91	50	63	63
(%)	(76)	(12)	(5)	(3)	(4)	

i-ii: Professional and managerial occupations
iii-v: Non-manual/manual/semi-skilled manual and unskilled manual

Table 3.2: Socioeconomic and offspring factors, by partner pattern of drinking (number of times 4+ units of alcohol in past month at 18 weeks gestation)

	Drinking patterns (partner)					
	None	1-2 days	3-4 days	5-10 days	>10 days	Total
Socioeconomic position (*n* = 7784)						
i-ii	509	495	598	849	590	3041
(%)	(17)	(16)	(20)	(28)	(19)	
iii-v	812	869	934	1211	917	4743

(%)	(17)	(18)	(20)	(26)	(19)	
Home ownership						
(*n* = 9616)						
Home owner	1254	1306	1454	1996	1410	7420
(%)	(17)	(18)	(20)	(27)	(19)	
Non-home owner	511	400	391	453	441	2196
(%)	(23)	(18)	(18)	(21)	(20)	
Marital status						
(*n* = 9671)						
Married	1416	1380	1536	1971	1408	7711
(%)	(18)	(18)	(20)	(26)	(18)	
Not married	360	339	320	485	456	1960
(%)	(18)	(18)	(16)	(25)	(23)	
Maternal education						
(*n* = 9271)						
University degree	237	237	250	377	250	1351
(%)	(18)	(18)	(19)	(28)	(19)	
No university degree	1455	1409	1531	1999	1526	7920
(%)	(18)	(18)	(19)	(25)	(19)	
Offspring sex						
(*n* = 9890)						
Male	931	904	958	1307	982	5082
(%)	(18)	(18)	(19)	(26)	(19)	
Female	902	850	937	1198	921	4808
(%)	(19)	(18)	(19)	(25)	(19)	
Parity (*n* = 9741)						
First born	727	768	882	1255	904	4536
(%)	(16)	(17)	(190	(28)	(20)	
2_{nd}+ born	1086	948	982	1213	976	5205
(%)	(21)	(18)	(19)	(23)	(19)	
Smoked during						
pregnancy (*n* = 9904)						
No	1458	1376	1492	1942	1403	7671
(%)	(19)	(18)	(19)	(25)	(18)	
Yes	384	377	408	560	504	2233
(%)	(17)	(17)	(18)	(25)	(23)	

Drug use during pregnancy (*n* = 9766)						
No	1789	1723	1869	2466	1878	9725
(%)	(18)	(18)	(19)	(25)	(19)	
Yes	12	9	5	9	6	41
(%)	(29)	(22)	(12)	(22)	(15)	
Depression 18 weeks gestation (*n* = 9517)						
No	1431	1406	1553	2082	1535	8007
(%)	(18)	(18)	(19)	(26)	(19)	
Yes	238	209	204	258	241	1150
(%)	(21)	(18)	(18)	(22)	(21)	

i-ii: Professional and managerial occupations
iii-v: Non-manual/manual/semi-skilled manual and unskilled manual

Table 3.3: Socioeconomic and offspring factors by maternal alcohol frequency at 18 weeks gestation

	Drinking patterns (maternal)					
	None	< 1 glass per week	1+ glass per week	1-2 glasses per day	3+ glass per day	Total
Socioeconomic position (*n* = 9905)						
i-ii	1565	1549	542	56	9	3721
(%)	(42)	(41)	(15)	(2)	(<1)	
iii-v	2847	2389	845	90	13	6184
(%)	(46)	(39)	(14)	(1)	(<1)	
Home ownership (*n* = 12683)						
Home owner	4197	3777	1280	133	16	9403
(%)	(45)	(40)	(14)	(1)	(<1)	
Non-home owner	1566	1156	470	67	21	3280
(%)	(48)	(35)	(14)	(2)	(1)	
Marital status (*n* = 12738)						
Married	4446	3856	1202	101	16	9621

(%)	(46)	(41)	(12)	(1)	(<1)	
Not married	1351	1109	540	98	19	3117
(%)	(43)	(36)	(17)	(3)	(<1)	
Maternal education (*n* = 12088)						
University degree	619	682	253	33	2	1589
(%)	(39)	(43)	(16)	(2)	(1)	
No university degree	4837	4055	1424	154	29	10499
(%)	(46)	(39)	(14)	(1)	(<1)	
Offspring sex (*n* = 13123)						
Male	3071	2597	972	116	24	6780
(%)	(45)	(38)	(14)	(2)	(<1)	
Female	2898	2486	849	94	16	6343
(%)	(46)	(38)	(13)	(1)	(<1)	
Parity (*n* = 12966)						
First born	2794	2103	777	104	21	5799
(%)	(48)	(36)	(13)	(2)	(<1)	
2nd+ born	3101	2926	1017	105	18	7167
(%)	(43)	(41)	(14)	(1)	(<1)	
Smoked during pregnancy (*n* = 13195)						
No	4695	3858	1202	98	11	9867
(%)	(39)	(37)	(19)	(3)	(<1)	
Yes	1307	1247	629	114	31	3328
(%)	(39)	(37)	(19)	(3)	(<1)	
Drug use during pregnancy (*n* = 13028)						
No	5902	5034	1788	204	40	12968
(%)	(46)	(39)	(14)	(2)	(<1)	
Yes	19	19	16	4	2	60
(%)	(32)	(32)	(27)	(7)	(3)	
Depression 18 weeks gestation (*n* = 12061)						
No	4721	4079	1423	147	25	13395
(%)	(45)	(39)	(14)	(1)	(<1)	

Yes	755	600	256	43	12	1666
(%)	(45)	(36)	(15)	(3)	(<1)	

i-ii: Professional and managerial occupations

iii-v: Non-manual/manual/semi-skilled manual and unskilled manual

Table 3.4: Socioeconomic and offspring factors by partner alcohol frequency at 18 weeks gestation

	Drinking frequency (maternal)					
	None	< 1 glass per week	1+ glass per week	1-2 glasses per day	3+ glass per day	Total
Socioeconomic position (*n* = 7721)						
i-ii	92	586	1562	603	173	3016
(%)	(3)	(19)	(52)	(20)	(60	
iii-v	201	1225	2436	639	204	4705
(%)	(4)	(26)	(52)	(14)	(4)	
Home ownership (*n* = 9520)						
Home owner	267	1653	3868	1237	334	7359
(%)	(4)	(22)	(53)	(17)	(5)	
Non-home owner	189	663	937	242	130	2161
(%)	(9)	(31)	(43)	(11)	(6)	
Marital status (*n* = 9574)						
Married	332	1827	3928	1217	328	7632
(%)	(4)	(24)	(51)	(16)	(4)	
Not married	128	509	896	270	139	1942
(%)	(7)	(26)	(46)	(14)	(7)	
Maternal education (*n* = 9191)						
University degree	39	229	687	294	95	1344
(%)	(3)	(17)	(51)	(22)	(7)	
No university degree	397	1993	3965	1140	352	7847

(%)	(5)	(25)	(51)	(15)	(4)	
Offspring sex (*n* = 9792)						
Male	255	1215	2525	802	234	5031
(%)	(5)	(24)	(50)	(16)	(5)	
Female	228	1183	2394	711	245	4761
(%)	(5)	(25)	(50)	(15)	(5)	
Parity (*n* = 9648)						
First born	205	990	2355	739	218	4507
(%)	(5)	(22)	(52)	(16)	(5)	
2nd+ born	271	1377	2487	749	257	5141
(%)	(5)	(27)	(48)	(15)	(5)	
Smoked during pregnancy (*n* = 9804)						
No	341	1787	3925	1217	326	7596
(%)	(4)	(24)	(52)	(16)	(4)	
Yes	145	613	997	299	154	2208
(%)	(7)	(28)	(45)	(14)	(7)	
Drug use during pregnancy (*n* = 9676)						
No	473	2350	4848	1495	469	9635
(%)	(5)	(24)	(50)	(16)	(5)	
Yes	3	12	20	3	3	41
(%)	(7)	(29)	(49)	(7)	(7)	
Depression 18 weeks gestation (*n* = 9065)						
No	348	1877	4091	1220	387	7923
(%)	(4)	(24)	(52)	(15)	(5)	
Yes	82	321	513	169	57	1142
(%)	(7)	(28)	(45)	(15)	(5)	

i-ii: Professional and managerial occupations

iii-v: Non-manual/manual/semi-skilled manual and unskilled manual

Maternal alcohol consumption and offspring depression

Individuals whose mothers consumed any alcohol at 18 weeks gestation were at increased odds of having a diagnosis of depression at age 18 (unadjusted OR = 1.18, 95% CI 1.03 to 1.34). After adjustment for socioeconomic and maternal behaviours these associations were attenuated only slightly (OR = 1.13, 95% CI 0.99 to 1.29, Table 3.5). Further adjustment for partner alcohol use strengthened the association slightly (OR = 1.17, 95% CI 1.02 to 1.34).

Table 3.5: Associations between maternal and partner alcohol frequency and offspring depression (CIS-R) at age 18 years

		Unadjusted		Adjusted[1]		Adjusted[2]		Adjusted[3]	
	n = 13480	OR (CI)	*P*	OR (CI)	*P*	OR (CI)	*P*	OR (CI)	*P*
Mothers	Never	1.00 (ref)	0.036[a]	1.00 (ref)	0.087[a]	1.00 (ref)	0.237[a]	1.00 (ref)	0.129[a]
	<1 glass per week	1.09 (0.88-1.36)		1.11 (0.89-1.39)		1.09 (0.87-1.37)		1.12 (0.90-1.41)	
	1+ glass per week	1.90 (0.88-1.60)		1.17 (0.86-1.60)		1.11 (0.81-1.52)		1.19 (0.87-1.63)	
	1-2 glasses per day	1.93 (0.92-4.03)		1.81 (0.84-3.87)		1.59 (0.73-3.46)		1.85 (0.83-4.11)	
	3+ glasses per day	5.34 (1.29-22.01)		4.91 (1.07-22.44)		4.06 (0.86-19.31)		4.45 (0.92-21.46)	
	Linear trend	1.18 (1.03-1.34)	0.015	1.17 (1.02-1.33)	0.024	1.13 (0.99-1.29)	0.075	1.17 (1.02-1.34)	0.025
Fathers	Never	1.00 (ref)	0.087[a]	1.00 (ref)	0.203[a]	1.00 (ref)	0.209[a]	1.00 (ref)	0.108[a]
	<1 glass per week	1.21 (0.69-2.12)		1.27 (0.71-2.26)		1.28 (0.71-2.29)		1.24 (0.69-2.22)	
	1+ glass per week	0.88 (0.48-1.60)		0.96 (0.51-1.78)		0.97 (0.51-1.81)		0.90 (0.48-1.71)	
	1-2 glasses per day	0.77 (0.41-1.45)		0.85 (0.44-1.65)		0.86 (0.44-1.67)		0.77 (0.39-1.53)	
	3+ glasses per day	0.97 (0.45-2.08)		0.99 (0.45-2.18)		0.97 (0.44-2.16)		0.85 (0.38-1.93)	
	Linear trend	0.88 (0.77-1.02)	0.086	0.90 (0.77-1.04)	0.161	0.90 (0.77-1.04)	0.154	0.87 (0.74-1.01)	0.071

[1]Adjusted for: socioeconomic position, income, home ownership, marital status, maternal education, sex, parity

[2]Adjusted for: socioeconomic position, income, home ownership, marital status, maternal education, sex, parity, maternal tobacco use during 1-3 months of pregnancy, maternal illicit drug use during 1-3 months of pregnancy, maternal depression 18 weeks gestation

[3]Adjusted for: socioeconomic position, income, home ownership, marital status, maternal education, sex, parity, maternal tobacco use during 1-3 months of pregnancy, maternal illicit drug use during 1-3 months of pregnancy, maternal depression 18 weeks gestation, how often partner consumed alcohol at 18 weeks gestation

[a]Wald test

Table 3.6: Associations between maternal and partner alcohol binge drinking and offspring depression (CIS-R) at age 18 years

		Unadjusted		Adjusted[1]		Adjusted[2]		Adjusted[3]	
	n = 13480	OR (CI)	*P*	OR (CI)	*P*	OR (CI)	*P*	OR (CI)	*P*
Mothers	None	1.00 (ref)	0.466[a]	1.00 (ref)	0.708[a]	1.00 (ref)	0.797[a]	1.00 (ref)	0.797[a]
	1-2 days	1.07 (0.76-1.52)		1.00 (0.70-1.42)		0.95 (0.67-1.37)		0.98 (0.68-1.41)	
	3-4 days	1.55 (0.98-2.43)		1.37 (0.85-2.20)		1.22 (0.75-1.98)		1.27 (0.78-2.06)	
	5-10 days	1.19 (0.57-2.48)		1.07 (0.51-2.27)		0.95 (0.44-2.03)		1.01 (0.47-2.16)	
	>10 days	0.89 (0.40-1.98)		0.76 (0.33-1.72)		0.68 (0.29-1.57)		0.71 (0.31-1.63)	
	Linear trend	1.06 (0.94-1.19)	0.329	1.01 (0.90-1.15)	0.831	0.97 (0.86-1.11)	0.697	0.99 (0.87-1.13)	0.884
Fathers	None	1.00 (ref)	0.348[a]	1.00 (ref)	0.378[a]	1.00 (ref)	0.354[a]	1.00 (ref)	0.363[a]
	1-2 days	0.85 (0.61-1.19)		0.87 (0.62-1.23)		0.87 (0.62-1.22)		0.87 (0.62-1.22)	
	3-4 days	0.74 (0.53-1.05)		0.76 (0.53-1.09)		0.76 (0.53-1.09)		0.76 (0.54-1.09)	
	5-10 days	0.89 (0.45-1.22)		0.92 (0.66-1.29)		0.91 (0.65-1.28)		0.91 (0.66-1.28)	
	>10 days	0.75 (0.53-1.06)		0.75 (0.52-1.07)		0.73 (0.51-1.06)		0.74 (0.51-1.06)	
	Linear trend	0.95 (0.88-1.03)	0.195	0.95 (0.88-1.03)	0.224	0.95 (0.87-1.03)	0.189	0.95 (0.87-1.03)	0.194

[1]Adjusted for: socioeconomic position, income, home ownership, marital status, maternal education, sex, parity

[2]Adjusted for: socioeconomic position, income, home ownership, marital status, maternal education, sex, parity, maternal tobacco use during 1-3 months of pregnancy, maternal illicit drug use during 1-3 months of pregnancy, maternal depression 18 weeks gestation

[3]Adjusted for: socioeconomic position, income, home ownership, marital status, maternal education, sex, parity, maternal tobacco use during 1-3 months of pregnancy, maternal illicit drug use during 1-3 months of pregnancy, maternal depression 18 weeks gestation, how often partner consumed alcohol at 18 weeks gestation

[a]Wald test

There was no clear evidence that the number of days when ≥4 alcoholic drinks were consumed over the last month at 18 or 32 weeks gestation was associated with offspring depression at age 18 (see Table 3.6 and Appendices 3.1).

In sensitivity analyses of offspring depression at age 24 there was no clear evidence that frequency mothers consumed alcohol at 18 weeks gestation was associated with offspring depression (unadjusted OR = 1.07, 95% CI 0.93 to 1.24) (see Table 3.7). The number of days when ≥4 alcoholic drinks were consumed over the last month at 18 weeks gestation was weakly associated with offspring depression at age 24 (unadjusted OR = 1.13, 95% CI 1.00 to 1.27). After adjustment for socioeconomic and maternal behaviours these associations did not persist (OR = 1.04, 95% CI 0.92 to 1.19, (see Appendices 3.3). The number of days when ≥4 alcoholic drinks were consumed over the last month at 32 weeks gestation was weakly associated with offspring depression at age 24 (OR = 1.28, CI 1.12 to 1.47). these associations were attenuated after adjustment for confounding influences (OR = 1.20, CI 1.03 to 1.40).

The findings from the full sample and complete case analyses did not differ substantially from the imputed analyses (see Appendices 3.3 to 3.11).

Partner alcohol consumption and offspring depression

Paternal alcohol use at 18 weeks gestation showed no clear evidence of association with offspring depression at age 18, for both frequency (unadjusted OR = 0.88, 95% CI 0.77 to 1.02, Table 3.5) and pattern of alcohol use (unadjusted OR = 0.95, 95% CI 0.94 to 1.19, see Table 3.6).

In sensitivity analyses of offspring depression at age 24, I found no clear evidence that offspring of mothers whose partners consumed any alcohol at 18 weeks gestation were at increased odds of having a diagnosis of depression at age 24 (see Tables 3.7 and 3.8). The findings from the full sample and complete case analyses did not differ substantially from the imputed analyses (see Appendices 3.3 to 3.11).

Patterns of missing data were also explored for the demographic variables, and responders and non-responders to the CIS-R depression questionnaire differed for all key demographic variables, such as socioeconomic position, income and education (see Table 3.9).

Table 3.7: Associations between maternal and partner alcohol frequency at 18 weeks and offspring depression (CIS-R) at age 24, imputed data

		Unadjusted		Adjusted[1]		Adjusted[2]		Adjusted[3]	
	n = 13480	OR (CI)	*P*	OR (CI)	*P*	OR (CI)	*P*	OR (CI)	*P*
Mothers	Never	1.00 (ref)	0.367[a]	1.00 (ref)	0.729[a]	1.00 (ref)	0.873[a]	1.00 (ref)	0.882[a]
	<1 glass per week	0.93 (0.74-1.16)		0.96 (0.76-1.21)		0.94 (0.75-1.19)		0.94 (0.74-1.19)	
	1+ glass per week	1.09 (0.81-1.46)		1.07 (0.79-1.45)		1.02 (0.75-1.39)		1.01 (0.74-1.37)	
	1-2 glasses per day	1.75 (0.84-3.62)		1.55 (0.72-3.31)		1.38 (0.64-2.97)		1.36 (0.63-2.94)	
	3+ glasses per day	1.71 (0.33-8.90)		1.28 (0.23-7.13)		1.09 (0.19-6.15)		1.08 (0.19-6.02)	
	Linear trend	1.07 (0.93-1.24)	0.353	1.06 (0.91-1.22)	0.465	1.03 (0.88-1.19)	0.727	1.02 (0.88-1.18)	0.768
Fathers	Never	1.00 (ref)	0.372[a]	1.00 (ref)	0.684[a]	1.00 (ref)	0.752[a]	1.00 (ref)	[a]
	<1 glass per week	0.72 (0.42-1.24)		0.78 (0.44-1.36)		0.78 (0.45-1.37)		0.78 (.044-1.36)	
	1+ glass per week	0.67 (0.39-1.15)		0.79 (0.46-1.37)		0.80 (0.46-1.39)		0.79 (0.46-1.37)	
	1-2 glasses per day	0.69 (0.38-1.25)		0.85 (0.47-1.56)		0.86 (0.47-1.57)		0.84 (0.46-1.54)	
	3+ glasses per day	0.92 (0.48-1.76)		1.03 (0.53-2.00)		1.02 (0.52-1.98)		1.00 (0.52-1.91)	
	Linear trend	0.98 (0.86-1.12)	0.733	1.03 (0.90-1.17)	0.706	1.02 (0.90-1.17)	0.740	1.02 (0.89-1.16)	0.790

Model [1]: socioeconomic position, income, home ownership, marital status, maternal education, gender, parity. Model [2]: socioeconomic position, income, home ownership, marital status, maternal education, gender, parity, maternal tobacco use during 1-3 months of pregnancy, maternal illicit drug use during 1-3 months of pregnancy, maternal depression 18 weeks gestation. Model [3]: socioeconomic position, income, home ownership, marital status, maternal education, gender, parity, maternal tobacco use during 1-3 months of pregnancy, maternal illicit drug use during 1-3 months of pregnancy, maternal depression 18 weeks gestation, how often partner consumed alcohol at 18 weeks gestation. [a]Wald test

Table 3.8: Associations between maternal and partner binge drinking at 18 weeks gestation and offspring depression (CIS-R) at age 24, imputed data

		Unadjusted		Adjusted[1]		Adjusted[2]		Adjusted[3]	
	n = 13480	OR (CI)	*P*	OR (CI)	*P*	OR (CI)	*P*	OR (CI)	*P*
Mothers	None	1.00 (ref)	0.349[a]	1.00 (ref)	0.746[a]	1.00 (ref)	0.823[a]	1.00 (ref)	0.817[a]
	1-2 days	1.03 (0.70-1.51)		0.92 (0.62-1.36)		0.87 (0.58-1.31)		0.88 (0.59-1.31)	
	3-4 days	1.40 (0.88-2.24)		1.21 (0.74-1.99)		1.12 (0.68-1.84)		1.13 (0.69-1.85)	
	5-10 days	1.15 (0.48-2.73)		0.98 (0.40-2.42)		0.91 (0.37-2.24)		0.92 (0.37-2.26)	
	>10 days	1.76 (0.94-3.30)		1.46 (0.76-2.81)		1.35 (0.69-2.65)		1.36 (0.69-2.69)	
	Linear trend	1.13 (1.00-1.27)	0.044	1.07 (0.94-1.21)	0.305	1.04 (0.91-1.19)	0.549	1.04 (0.92-1.19)	0.515
Fathers	None	1.00 (ref)	0.727[a]	1.00 (ref)	0.859[a]	1.00 (ref)	0.865[a]	1.00 (ref)	[a]
	1-2 days	0.85 (0.58-1.23)		0.88 (0.60-1.29)		0.88 (0.60-1.29)		0.87 (0.60-1.27)	
	3-4 days	0.79 (0.55-1.12)		0.84 (0.59-1.20)		0.83 (0.58-1.19)		0.82 (0.58-1.17)	
	5-10 days	0.91 (0.66-1.25)		0.97 (0.70-1.35)		0.96 (0.69-1.33)		0.94 (0.68-1.31)	
	>10 days	0.93 (0.65-1.33)		0.95 (0.66-1.37)		0.93 (0.64-1.34)		0.90 (0.63-1.31)	
	Linear trend	0.99 (0.92-1.07)	0.847	1.00 (0.92-1.08)	0.989	0.99 (0.92-1.08)	0.872	0.99 (0.91-1.07)	0.771

Model [1]: socioeconomic position, income, home ownership, marital status, maternal education, gender, parity. Model [2]: socioeconomic position, income, home ownership, marital status, maternal education, gender, parity, maternal tobacco use during 1-3 months of pregnancy, maternal illicit drug use during 1-3 months of pregnancy, maternal depression 18 weeks gestation. Model [3]: socioeconomic position, income, home ownership, marital status, maternal education, gender, parity, maternal tobacco use during 1-3 months of pregnancy, maternal illicit drug use during 1-3 months of pregnancy, maternal depression 18 weeks gestation, how often partner consumed alcohol at 18 weeks gestation. [a]Wald test

Table 3.9: Comparison of responders and non-responders to the CIS-R depression questionnaire at 18 years by key demographic variables

	No data on outcome	Data on outcome	χ^2	Pvalue
Socioeconomic position				
i-ii	1632 (43%)	2140 (57%)	131.1	<0.001
iii-v	2020 (32%)	4303 (68%)		
Income				
0. Highest	930 (46%)	1077 (54%)	210.9	<0.001
1	854 (43%)	1129 (57%)		
2	788 (40%)	1184 (60%)		
3	709 (36%)	1259 (64%)		
4. Lowest	514 (38%)	1474 (74%)		
Home ownership				
Home owner	3538 (36%)	6325 (64%)	414.1	<0.001
Non-home owner	634 (18%)	2975 (82%)		
Marital status				
Married	3438 (34%)	6683 (66%)	153.3	<0.001
Not married	771 (23%)	2638 (77%)		
Maternal education				
University degree	792 (49%)	815 (51%)	212.8	<0.001
No university degree	3356 (31%)	7501 (69%)		
Offspring sex				
Male	1995 (26%)	5621 (74%)	154.5	<0.001
Female	2567 (36%)	4639 (64%)		
Parity				
First born	2010 (34%)	3852 (66%)	32.6	<0.001
2_{nd}+ born	2143 (30%)	5092 (70%)		
Smoked during pregnancy				
No	3536 (35%)	6435 (65%)	279.0	<0.001
Yes	671 (20%)	2688 (80%)		
Drug use during pregnancy				
No	4154 (32%)	8851 (68%)	9.4	0.002
Yes	9 (14%)	55 (86%)		

Depression 18 weeks gestation				
No	3480 (33%)	6966 (67%)	50.1	<0.001
Yes	416 (25%)	1272 (75%)		
Maternal alcohol frequency				
Never drink	254 (33%)	527 (67%)	47.1	<0.001
<once a week	1411 (40%)	2111 (60%)		
At least once a week	1392 (43%)	1836 (57%)		
1-2 units nearly every day	612 (47%)	698 (53%)		
3+ units per day	61 (41%)	88 (59%)		
Partner alcohol frequency				
Never drink	61 (39%)	95 (61%)	12.0	0.018
<once a week	443 (46%)	517 (54%)		
At least once a week	912 (49%)	968 (51%)		
1-2 units nearly every day	592 (52%)	557 (48%)		
3+ units per day	184 (48%)	203 (52%)		

i-ii: Professional and managerial occupations
iii-v: Non-manual/manual/semi-skilled manual and unskilled manual

3.4 Discussion

I investigated the associations between maternal alcohol consumption in pregnancy (frequency and pattern) and offspring depression in a population-based study. The results suggest that the frequency mothers consumed alcohol during pregnancy at 18 weeks gestation was associated with offspring depression at age 18, and indicate a linear pattern between the amount of alcohol mothers drink in pregnancy and offspring risk of depression. However, the strength of association was attenuated after controlling for confounding influences, which suggests that much of the associations found may actually be due to the socioeconomic confounders that were adjusted for in the models. Maternal pattern of alcohol consumption (binge drinking) at 18 weeks showed no clear evidence of association with offspring depression, however, maternal binge drinking at 32 weeks gestation showed an association with offspring depression at 24 years. After adjustment for confounding influences, these associations were also attenuated. As maternal binge drinking at 32 weeks gestation was only associated with offspring depression at age 24, and not the earlier age also of 18, this could suggest other unmeasured environmental influences could be influencing depression at a later age. For partner alcohol consumption

there was no clear evidence of association of either the frequency or pattern of alcohol use and offspring depression at age 18 or 24. This negative control analysis suggests that the associations shown for maternal alcohol consumption in pregnancy and offspring depression may be causal. However, these findings may not be robust as the associations were attenuated or removed altogether after adjustment for confounding influences, suggesting they may actually be driven by confounding factors.

The use of a negative control comparison of paternal drinking in pregnancy provides some support for the possibility that the observations observed may be causal. By using a long follow up for the main outcome (at age 18), my findings suggest that any associations shown within the offspring are likely to persist throughout childhood and into adulthood. Although I also found that the associations were attenuated for frequency alcohol was consumed when measuring prenatal alcohol use against offspring depression at a later timepoint (age 24), the direction of association remained the same. The sensitivity analysis also suggested that maternal binge drinking at 32 weeks gestation is associated with offspring depression at age 24. These results at age 24, were less precisely estimated, potentially due to sample attrition, and it is possible that a small true association exists which, if causal, would potentially be of public health importance.

Although the associations I observed are relatively weak, they may nevertheless be important at a population level, particularly as depression is a common mental health disorder affecting more than 300 million people globally (WHO, 2017). The population-attributable fraction (PAF) was calculated for each analysis model using the UK prevalence of alcohol use during pregnancy (O'Keeffe et al., 2015) for the comparison numerators and denominators. The PAFs ranged between 0.05 to 0.15 for maternal, and 0.04 to 0.05 for partners, for the contribution of alcohol use during pregnancy on offspring depression. This suggests that if the associations observed are causal and precisely estimated, the percentage of depression cases that are preventable by almost removing alcohol consumption during pregnancy ranges between 4% and 15%, depending on which estimate this is based on. Such findings have implications for women trying to conceive or who may not be aware that they are already pregnant, when the fetus is most likely to be exposed to alcohol (Floyd, Decouflé, & Hungerford, 1999). The findings therefore provide support for guidelines recommending complete abstinence from alcohol during pregnancy, or for women trying to conceive.

However, if must be noted that the ALSPAC cohort may not be generalisable to the whole of the UK as this is a population-based cohort consisting of participants only from the area of Avon during a fixed time period. Participants within ALSPAC have also been shown to consist of individuals from a more advantageous socio-demographic background (e.g. more likely to own their house and own vehicle, currently married)

compared to the whole of Great Britain, as well consisting of predominantly white mothers (Fraser et al., 2013). An advantage of the ALSPAC cohort, where recruitment occurred during 1990-1991, is that attitudes in the UK towards drinking in pregnancy were likely to have been different to current day with less stigma associated with drinking in pregnancy, meaning that mothers could have been more likely to truthfully report alcohol consumption. However, underreporting of alcohol use may still have occurred if mothers were not aware they were pregnant until later stages of pregnancy, therefore misrepresenting the true level of alcohol exposure as the alcohol exposures rely on valid self-report. If alcohol use was underreported, the findings I observed are likely to be more conservative and a larger association could have been shown if there was a more biologically valid way to assess maternal alcohol consumption.

There are limitations that should be considered when interpreting these results. Firstly, there is sample attrition from enrolment to the outcome measurement at age 18. Characteristics between responders and non-responders in the ALSPAC study could therefore have caused selection bias. However, as the complete case analyses and those using the imputed dataset do not differ substantially, selection bias is unlikely to have affected the reported associations. Previous studies investigating biases within the ALSPAC cohort have found the strength of associations to not be greatly affected by selection bias and sample attrition (Wolke et al., 2009). Secondly, the associations may also be due to a shared genetic risk for depression, which is expressed as different phenotypes in mothers and offspring, with increased alcohol use for mothers and increased depression in their offspring. However, genetic data were not included in this analysis to be able to test this. Third, the influence of the postnatal environment was not included within the analyses conducted. A postnatal measure of parental depression was not included, and only perinatal depression was measured due to its potential influence on increased alcohol consumption. Postnatal depression has been associated with an increased risk of adverse offspring outcomes such as increased internalising disorders (Verbeek et al., 2012). and potentially this could have influenced offspring depression. As this chapter included an older offspring age group nearer to age 18, it is likely that the offspring have begun to consume alcohol themselves. There is a complex relationship between internalising disorders and alcohol use (Kushner, Abrams, & Borchardt, 2000) with increased alcohol shown to be a risk factor for internalising disorders (Kushner, Sher, & Erickson, 1999).

This chapter highlights the potentially long-lasting detrimental effects of maternal alcohol consumption in pregnancy on offspring mental health. Although the associations I observed are small, they may nevertheless be important at a population level. The negative control comparison of paternal alcohol use during gestation provides some

evidence that the associations found for maternal PAE may be causal. However, further research is needed to determine with greater confidence whether these associations are indeed causal, and the result of intrauterine exposure. This may require the use of other methodological approaches, such as Mendelian randomization, and sibling comparisons with offspring discordant for maternal alcohol consumption in pregnancy.

3.5 Chapter Summary

In this chapter I investigated the associations between both maternal and partner PAE and offspring depression at ages 18 and 24, in a negative control design. Overall, the main findings indicate that the amount of alcohol mothers consumed at 18 weeks gestation is associated with offspring depression, however, as the associations were attenuated after adjustment for potential confounding influences this suggests that some of the associations are due to socioeconomic characteristics, such as income or maternal education. As partner PAE was not associated with offspring depression, this suggests an intrauterine effect of maternal alcohol use only. Whilst the findings from this chapter suggest a teratogenic effect, the current chapter does not evaluate the possible influences of parental alcohol exposure during their offspring's childhood (postnatally) on offspring mental health. In the next chapter I continue to investigate parental alcohol use, but this time measuring mothers and partners alcohol use when offspring are older. This will help investigate if postnatal alcohol use is confounding the results shown within Chapter Three.

Chapter 4 Postnatal alcohol use and offspring mental health and behavioural problems

The findings presented in Chapter 3 highlight differences between the potential influences of maternal and partner alcohol use on offspring mental health problems, namely that maternal alcohol use during pregnancy was associated with offspring depression at age 18 and 24, while partner alcohol use during pregnancy was not. This suggests an intrauterine effect of maternal alcohol use. However, I have yet to test the association of parental drinking during the child's upbringing on their mental health. The following chapter aims to test if parental drinking postnatally is associated with offspring depression and behavioural problems, to help investigate if postnatal alcohol use may be confounding the results shown in Chapter Three. Removing biological routes to mental health, will investigate the association of postnatal alcohol use and offspring mental health as well as investigate if there are any additional environmental explanations of PAE being associated with offspring mental health. However, unlike Chapter Three a negative control analysis cannot be used in this chapter to assess causality. This is because within Chapter Three it was biologically impossible for partners alcohol use during pregnancy to affect offspring's intrauterine development. Yet, in the current chapter partners drinking at age 5 could feasibly affect offspring development through environmental exposures. Therefore, both maternal and partner alcohol use postnatally are included as exposures to account for confounding influences. Before extending the methodologies used to include genetic variants (for an alcohol exposure) and longitudinal repeated measures (for mental health outcomes) within Chapters 5 and 6, I aimed to test if the associations shown between maternal alcohol use and offspring mental health persisted for postnatal alcohol exposures.

4.1 Introduction

Parental alcohol use has been associated with negative offspring outcomes such as decreased IQ, behavioural problems and offspring mental health as previously discussed (Easey et al., 2019; Larkby et al., 2011; Zuccolo et al., 2013). However, many studies that have investigated parental alcohol use, have focused on prenatal effects. As shown in Chapter Two there are methodological challenges when studying prenatal alcohol exposure. Such challenges are the vast differences between studies in how prenatal alcohol use is measured, and the use of retrospective reports. A main problem in measuring maternal PAE is the potential for underreporting alcohol amounts potentially

due to fear of stigma. Previous research has shown alcohol consumption during pregnancy is likely to be under-reported, meaning PAE levels may be underestimated (Alvik, Haldorsen, Groholt, & Lindemann, 2006). Potentially, parents could be more likely to accurately report alcohol use at periods outside of pregnancy such as during childhood. Uncertainty also remains if these negative outcomes previously found to be associated with parental alcohol use are due to alcohol exposures during pregnancy, or environmental alcohol exposures after birth. How confounding structures may influence these pathways is also a major challenge. Higher SES has been linked with a range of positive health outcomes (Pedersen & Soest, 2017; Zimmer, Hanson, & Smith, 2016). Differences have also been shown between different SES groups for the quantity and pattern of alcohol consumption, with higher SES groups likely to drink more frequently, and lower SES groups more likely to drink heavier quantities (Casswell, Pledger, & Hooper, 2003; Huckle, You, & Casswell, 2010). Such confounding influences could actually be what is explaining associations shown for parental alcohol use and offspring outcomes.

Previous research investigating postnatal alcohol use has indicated that children of parents with alcohol use disorder (AUD) are at greater risk of externalising (Hussong, Huang, Curran, Chassin, & Zucker, 2010) and internalising (Hussong et al., 2008) disorders. The most drinks consumed in a 24-hour period is a validated alcohol use disorder phenotype, and has been shown that the maximum alcoholic drinks consumed by mothers in one 24-hour period is associated with increased offspring mental health problems in adolescence (Malone, McGue, & Iacono, 2010). Similarly to chapter three, studies have also sought to disentangle the separate influences of maternal and paternal postnatal alcohol use on offspring outcomes (Rognmo, Torvik, Ask, Røysamb, & Tambs, 2012). Rognmo and colleagues investigated both maternal and paternal alcohol use independently, finding maternal alcohol use to be the main predictor of offspring mental distress with paternal alcohol use showing a weak effect with limited precision. However, this study used parental alcohol abuse as the exposure of interest and is therefore more reflective of individuals with alcohol use disorder. A recent scoping review investigated the already available literature on parental alcohol use and adverse offspring outcomes, including any measures of alcohol use (Rossow, Felix, Keating, & McCambridge, 2016). Rossow and colleagues found that parental alcohol use was associated with offspring harm across a range of measures in two thirds of the included studies. However, outcomes measuring internalising problems accounted for only 18% of the included studies, and externalising disorders were measured for 12%, with adolescent alcohol use being the most common outcome measure studied. Mahedy et al (2017) also sought to

investigate both maternal and partner alcohol use, using exposures of heavy (binge) drinking and total alcohol amount (Mahedy et al., 2017). In contrast to previous studies, Mahedy and colleagues found a lack of evidence for an association of parental postnatal alcohol use and offspring depressive symptoms or conduct disorder. This may instead be due to measuring light to moderate alcohol levels instead of heavy alcohol use.

The aim of this chapter is to is to extend my work from Chapter Three to investigate the association of parental alcohol use (this time postnatally) and offspring mental health in late adolescence, again measuring both maternal and partner alcohol consumption. By removing the biological routes to offspring outcomes (e.g. intrauterine exposure) I can then be able to investigate if there are any environmental associations of parental drinking. It is likely that the adjusted models will show weaker associations as they are likely to be driven by confounding structures.

4.2 Methods

4.2.1 Sample

The ALSPAC cohort was again used in this chapter. A cohort description is given in Chapter Three.

4.2.2 Measures

Exposures. Parental alcohol consumption was measured when the offspring were 5 years old to allow comparison of the same available exposure measure for mothers and partners whilst maintaining sample size, by: 1) Frequency of drinking: mothers were asked the frequency and amount of alcohol they and their partners consume. Response categories were given as never, <1 glass per week, 1+ glass per week, 1-2 glasses a day, 3-9 glasses a day and ≥10 glasses a day. For the analyses, the last two categories were combined to 3+ glasses a day. 2) Parental binge drinking when offspring were 5 years old were measured by mothers being asked separately the number of days in the past month, they and their partner had consumed four or more units of alcohol. Response categories were none, 1-2 days, 3-4 days, 5-10 days, >10 days, and every day. For our analyses, the last two categories were combined to >10 days. Separate measures were recorded for maternal and partner frequency and pattern.

Outcomes. Depression in offspring was measured at age 18 using the computerised version of the CIS-R which is validated in reliability studies (Lewis, Pelosi, Araya, & Dunn, 1992). The CIS-R is a computerised interview consisting of questions that are scored between 0-5, with 5 depicting greater levels of depressive symptoms. These scores were then totalled, and scores greater than 12 indicated a clinical level of depression

according to ICD-10 criteria and were used as a binary measure of depression. The Strength and Difficulties Questionnaire (SDQ) (Goodman, 1997) is a reliable and validated parent-reported behavioural screening questionnaire (Goodman, 2001; Woerner et al., 2004), measuring hyperactivity, conduct-problems, emotional symptoms, peer problems and prosocial behaviour using 5 subscales ranging from 0-10, when offspring were 17 years old. Higher scores on each subscale indicate greater problems for all subscales, except for the pro-social behaviour subscale which is reverse scored. The first four subscales are then used to create a total difficulties subscale (ranging from 0-40). Offspring depression as measured by the CIS-R, and the total problem score from the SDQ, as well the subscales of hyperactivity, emotional symptoms and conduct problems were included in the current study as the main outcomes of interest. Validity and reliability of the SDQ have been calculated in previous studies, with internal consistency of the total problems scale and only the hyperactivity subscale showing a Cronbach's alpha coefficient ≥0.7, which is recommended for screening instruments (Mieloo et al., 2012). Validity of the parent rated SDQ has also been measured by calculating the Pearson correlation with the CBCL measure. The SDQ subscales have shown substantial correlations with other CBCL scales, and these indicate satisfactory reliability and validity of the total scores, however, there are concerns for the reliability of the SDQ subscales (Mieloo et al., 2012). Internal consistency and external validity for each subscale of the SDQ are shown in Appendices 6.2-6.3. The SDQ is treated as a continuous variable within this chapter however, for reference for the severity of each continuous score, the scores can also be divided into a three-fold classification to show severity of symptoms (Goodman, 1997) (Table 4.1).

Table 4.1 Categorising SDQ scores for 4-17 year olds

	Normal	Borderline	Abnormal
Total difficulties	0-13	14-16	17-40
Emotional problem score	0-3	4	5-10
Conduct Problem score	0-2	3	4-10
Hyperactivity score	0-5	6	7-10

Confounders. Potential confounding factors associated with parental alcohol consumption and offspring mental health disorders were included in the analysis. These confounders were kept the same as those used in Chapter Three, to keep the models similar across chapters. As described in Chapter Three, these consisted of socio-economic

variables, social factors, as well as maternal health behaviours added within separate adjusted models. Mother's socioeconomic position (professional/managerial or other) measured during pregnancy, income (divided into quintiles) measured at age 3 and 4 years, home ownership (mortgage/non-mortgage) measured at 8 weeks gestation, maternal education (university degree/<university degree), marital status (married or not) measured at 8 weeks gestation, sex, parity (first born, 2+ born), maternal tobacco during pregnancy (yes/no), illicit drug use during pregnancy (yes/no), and maternal depression at 18 weeks gestation (scores >12 highly associated with a diagnosis of depression) measured by the Edinburgh Postnatal Depression Scale (EPDS) (Cox et al., 1996), and maternal polygenic risk score (PRS) for depression. The maternal PRS for depression was calculated using single-nucleotide polymorphisms (SNPs) for depression identified in a genome wide association study of major depressive disorder (MDD) (Wray et al., 2018).

4.2.3 Statistical analyses

Distributions of confounders and maternal and partner alcohol patterns were investigated. Within the main analyses, I used linear regression to investigate associations between maternal and partner alcohol frequency and pattern when offspring were 5 years old, and all included behavioural subscales from the SDQ and the derived total problem score at 17 years. I used logistic regression to investigate associations between maternal and partner alcohol frequency and pattern (offspring aged 5 years), and a diagnosis of depression (CIS-R) at 18 years of age. Comparisons were made between the never drank controls in each alcohol exposure and each alcohol frequency/pattern group. The impact of confounders on these associations was explored by comparing unadjusted estimates to those adjusted for all given confounders except for maternal PRS for depression (model 1), and those further adjusted for maternal PRS for depression (model 2). Analyses were conducted using Stata version 15.1.

Similarly to Chapter Three, the impact of confounders on these associations was explored by comparing unadjusted estimates to those adjusted for socioeconomic variables , and variables of maternal behaviour during pregnancy (e.g. other drug use during pregnancy/self-reported maternal depression in pregnancy) (adjusted model 1), and in a final model adjusting for maternal PRS for depression (adjusted model 2).

Sensitivity analyses. Patterns of missing data were explored for exposures, outcomes and confounders. An imputed dataset was then created using multiple imputation by chained equations (MICE) as a sensitivity analyses to account for the high amount of missing data. Multiple imputation by chained equation was used to generate a maximum dataset

to account for missing data. Details of this method have previously been given in Chapter Three.

4.3 Results

4.3.1 Sample characteristics

When offspring were 5 years old, 91% of mothers (8,227 of 9,011) and 97% of partners (9,353 of 9,841) reported consuming alcohol. A total of 69% of mothers (5,233 of 8,917) and 84% of partners (3,787 of 4,518) reported consuming 4 or more units of alcohol on at least 1-2 days in the past month (see Figure 4.1). Mother, partner and offspring characteristics included in these analyses are presented in Table 4.1.

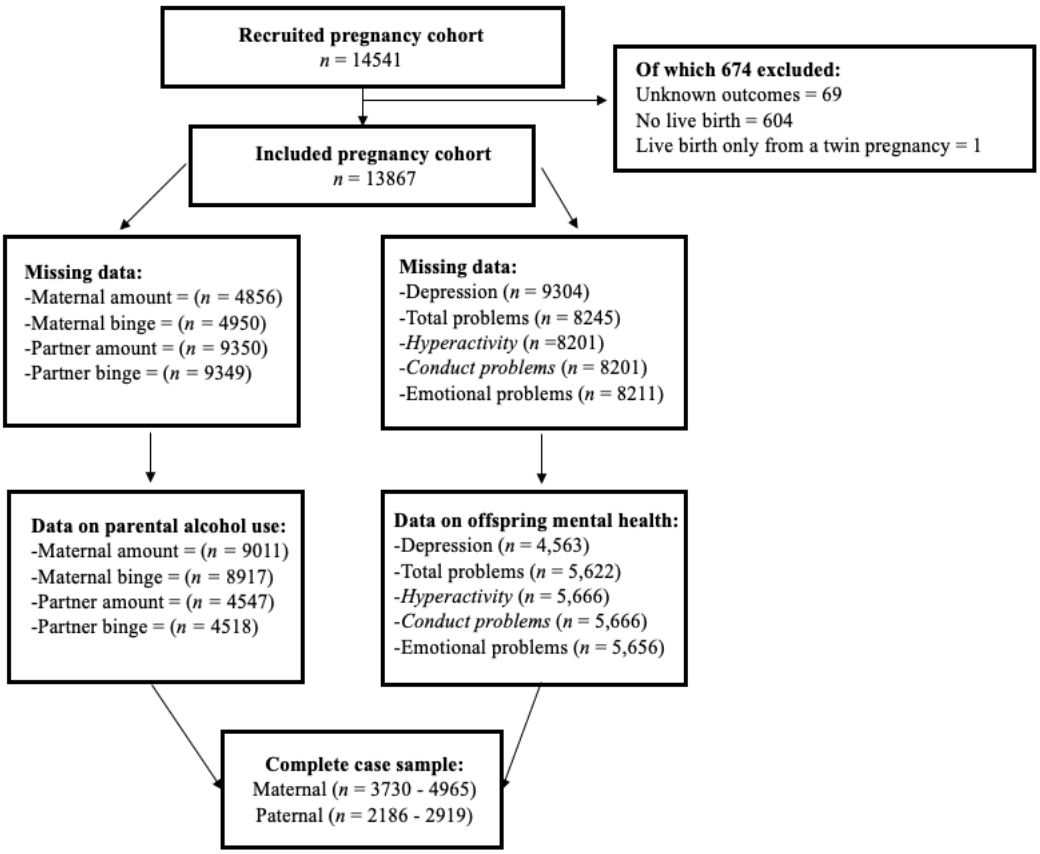

Figure 4.1: Flow chart of the mothers, partners and offspring included in the final sample

Table 4.2: Maternal and partner socioeconomic factors by frequency of alcohol consumption

	Alcohol frequency				
	None	1-2 <Once a week	At least once a week	1-2 units every day	At least 3 glasses per day
Maternal alcohol frequency					
Socioeconomic position					
i-ii	175	897	1218	634	61
(%)	(6)	(30)	(41)	(21)	(2)
iii-v	399	1914	1518	509	63
(%)	(9)	(43)	(35)	(12)	(1)
Income					
0. Highest	73	456	745	453	44
(%)	(4)	(26)	(42)	(25)	(3)
1	99	568	724	309	34
(%)	(6)	(33)	(42)	(18)	(2)
2	141	720	608	187	23
(%)	(9)	(43)	(36)	(11)	(1)
3	183	724	517	166	17
(%)	(12)	(45)	(32)	(10)	(1)
4. Lowest	204	773	389	112	22
(%)	(14)	(52)	(26)	(7)	(1)
Home ownership					
Home owner	486	2629	2643	1132	124
(%)	(7)	(37)	(38)	(16)	(2)
Non-home owner	268	791	514	157	25
(%)	(16)	(45)	(29)	(9)	(1)
Marital status					
Married	555	2672	2618	1075	108
(%)	(8)	(38)	(37)	(15)	(2)

Not married	204	766	568	218	40
(%)	(11)	(43)	(32)	(12)	(2)
Maternal education					
University degree	57	300	575	347	40
(%)	(4)	(23)	(44)	(26)	(3)
No university degree	690	3103	2584	943	108
(%)	(9)	(42)	(35)	(13)	(1)
Offspring sex					
Male	408	1849	1662	675	75
(%)	(9)	(40)	(36)	(14)	(2)
Female	376	1678	1575	638	75
(%)	(9)	(39)	(36)	(15)	(2)
Parity					
First born	344	1549	1462	602	60
(%)	(9)	(39)	(36)	(15)	(1)
2nd+ born	413	1858	1685	680	85
(%)	(9)	(39)	(36)	(14)	(2)
Smoked during pregnancy					
No	548	2681	2636	1051	96
(%)	(8)	(38)	(38)	(15)	(1)
Yes	225	769	555	251	52
(%)	(12)	(42)	(30)	(14)	(3)
Drug use during pregnancy					
No	758	3395	3148	1291	146
(%)	(9)	(39)	(36)	(15)	(2)
Yes	5	11	7	3	2
(%)	(18)	(39)	(25)	(11)	(7)
Depression 18 weeks gestation					
No	594	2792	2641	1091	104

(%)	(8)	(39)	(37)	(15)	(1)
Yes	97	402	308	122	32
(%)	(10)	(42)	(32)	(13)	(3)
Partner alcohol frequency					
Socioeconomic position					
i-ii	42	292	699	561	181
(%)	(2)	(16)	(40)	(32)	(10)
iii-v	82	499	913	469	166
(%)	(4)	(23)	(43)	(22)	(8)
Income					
0. Highest	24	126	460	408	124
(%)	(2)	(11)	(40)	(36)	(11)
1	27	163	454	282	92
(%)	(3)	(16)	(45)	(28)	(9)
2	23	207	366	223	61
(%)	(3)	(24)	(42)	(25)	(7)
3	33	227	297	115	52
(%)	(5)	(31)	(41)	(16)	(7)
4. Lowest	39	191	188	69	39
(%)	(7)	(36)	(36)	(13)	(7)
Home ownership					
Home owner	102	745	1582	1026	329
(%)	(3)	(20)	(42)	(27)	(9)
Non-home owner	52	189	276	104	55
(%)	(8)	(28)	(41)	(15)	(8)
Marital status					
Married	130	794	1605	997	319
(%)	(3)	(21)	(42)	(26)	(8)

Not married	25	149	263	145	67
(%)	(4)	(23)	(41)	(22)	(10)
Maternal education					
University degree	16	104	318	321	98
(%)	(2)	(12)	(37)	(37)	(11)
No university degree	136	836	1540	806	285
(%)	(4)	(23)	(43)	(22)	(8)
Offspring sex					
Male	86	496	960	622	198
(%)	(4)	(21)	(41)	(26)	(8)
Female	70	465	927	528	190
(%)	(3)	(21)	(43)	(24)	(9)
Parity					
First born	62	416	917	564	185
(%)	(3)	(19)	(43)	(26)	(9)
2nd+ born	91	523	930	572	193
(%)	(4)	(23)	(40)	(25)	(8)
Smoked during pregnancy					
No	116	749	1594	995	308
(%)	(3)	(20)	(42)	(26)	(8)
Yes	38	198	277	149	76
(%)	(5)	(27)	(38)	(20)	(10)
Drug use during pregnancy					
No	151	931	1848	1136	381
(%)	(3)	(21)	(42)	(26)	(9)
Yes	1	4	3	1	1
(%)	(10)	(40)	(30)	(10)	(10)
Depression 18 weeks gestation					
No	118	766	1580	989	315

(%)	(3)	(20)	(42)	(26)	(8)
Yes	22	101	154	87	42
(%)	(5)	(25)	(38)	(21)	(10)

i-ii: Professional and managerial occupations
iii-v: Non-manual/manual/semi-skilled manual and unskilled manual

Table 4.2 indicates that within each socioeconomic status level (i-ii; iii-v) mothers and partners from low socioeconomic backgrounds are more likely to have a lower frequency of alcohol consumption, with higher proportions in the alcohol consumption groups of never drinkers or drinking 1-2 units once or twice a week. Mothers and partners with a higher socioeconomic background have greater proportions of higher frequencies of alcohol consumption (1-2 units every day; 3+ units) within each SES group. The frequency of alcohol consumed within confounders is similar for mothers and partners, with the highest proportion mainly shown to be for consuming alcohol at least once a week.

4.3.2 Frequency of alcohol use

Maternal

Univariate analyses showed there was a negative association between maternal consumption of any alcohol when offspring were 5 years old and offspring total problems at age 17 (unadjusted coefficient -0.25, 95% CI -0.40 to -0.10). Negative associations were observed for all amounts of alcohol mothers consumed (Table 4.3). After adjustment for socioeconomic factors, maternal self-reported depression and substance use (tobacco and illicit drugs) during pregnancy, and maternal PRS for MDD these associations were attenuated (fully adjusted coefficient -0.12, 95% CI -0.33 to 0.09), and the statistical evidence for an association weakened considerably.

Table 4.3: Associations between maternal and partner alcohol frequency and offspring total problem score (SDQ) at age 17 years

	Unadjusted (*n* = 4927)		Adjusted[1] (*n* = 3779)		Adjusted[2] (*n* = 2683)	
	Coef (95% CI)	*p*	Coef (95% CI)	*p*	Coef (95% CI)	*p*
Mothers:						
Never	(ref)	0.0001[a]	(ref)	0.079[a]	1.00 (ref)	0.251[a]
<Once a week	-0.76 (-1.30, -0.23)		-0.75 (-1.37, -0.13)		-0.80 (-1.57, -0.03)	
At least once a week	-1.22 (-1.30, -0.70)		-0.82 (-1.44, -0.20)		-0.90 (-1.67, -0.13)	
1-2 units every day	-0.93 (-1.51, -0.34)		-0.62 (-1.30, 0.61)		-0.76 (-1.60, 0.82)	
At least 3 glasses pd	-1.15 (-2.25, -0.05)		-1.33 (-2.57, -0.08)		-0.91 (-2.39, 0.58)	
Linear trend	-0.25 (-0.40, -0.10)	0.001	-0.12 (-0.29, 0.06)	0.190	-0.12 (-0.33, 0.09)	0.262
	Unadjusted (*n* = 2901)		Adjusted[1] (*n* = 2318)		Adjusted[2] (*n* = 1689)	
Partners:						
Never	(ref)	0.015[a]	(ref)	0.816[a]	(ref)	0.832[a]
<Once a week	-0.14 (-1.26, 0.98)		-0.05 (-1.30, 1.19)		-0.74 (-2.30, 0.81)	
At least once a week	-0.76 (-1.84, 0.33)		-0.30 (-1.50, 0.89)		-0.87 (-2.38, 0.63)	
1-2 units every day	-0.94 (-2.04, 0.17)		-0.28 (-1.50, 0.93)		-0.88 (-2.41, 0.65)	
At least 3 glasses pd	-0.78 (-1.97, 0.41)		-0.43 (-1.74, 0.88)		-0.83 (-2.46, 0.80)	
Linear trend	-0.26 (-0.44, -0.08)	0.004	-0.10 (-0.30, 0.09)	0.299	-0.08 (-0.31, 0.16)	0.523

[1] Adjusted for: socioeconomic position, income, home ownership, marital status, maternal education, sex, parity, maternal tobacco use during 1-3 months of pregnancy, maternal illicit drug use during 1-3 months of pregnancy, maternal depression 18 weeks gestation

[2]Adjusted for: socioeconomic position, income, home ownership, marital status, maternal education, sex, parity, maternal tobacco use during 1-3 months of pregnancy, maternal illicit drug use during 1-3 months of pregnancy, maternal depression 18 weeks gestation, maternal PRS for major depressive disorder

[a]Omnibus p-value

Table 4.4: Associations between maternal alcohol frequency and offspring hyperactivity (SDQ) at age 17 years

	Unadjusted (n = 4965)		Adjusted[1] (n = 3804)		Adjusted[2] (n = 2700)	
	Coef (95% CI)	*p*	Coef (95% CI)	*p*	Coef (95% CI)	*p*
Never	(ref)	0.013[a]	(ref)	0.140[a]	(ref)	0.281[a]
<Once a week	-0.29 (-0.53, -0.05)		-0.31 (-0.59, -0.04)		-0.36 (-0.69, -0.02)	
At least once a	-0.41 (-0.65, -0.17)		-0.24 (-0.51, 0.03)			
week					-0.31 (-0.65, 0.02)	
	-0.29 (-0.56, -0.03)		-0.16 (-0.46, 0.14)			
1-2 units every day					-0.23 (-0.59, 0.14)	
At least 3 glasses	-0.46 (-0.94, 0.03)		-0.43 (-0.98, 0.11)			
pd					-0.24 (-0.89, 0.41)	
Linear trend	-0.07 (-0.14, -0.01)	0.031	-0.002 (-0.78, 0.07)	0.968	-0.002 (-0.09, 0.90)	0.974

[1] Adjusted for: socioeconomic position, income, home ownership, marital status, maternal education, sex, parity, maternal tobacco use during 1-3 months of pregnancy, maternal illicit drug use during 1-3 months of pregnancy, maternal depression 18 weeks gestation

[2]Adjusted for: socioeconomic position, income, home ownership, marital status, maternal education, sex, parity, maternal tobacco use during 1-3 months of pregnancy, maternal illicit drug use during 1-3 months of pregnancy, maternal depression 18 weeks gestation, maternal PRS for major depressive disorder

[a]Omnibus p-value

Table 4.5: Associations between maternal alcohol frequency and offspring emotional symptom score (SDQ) at age 17 years

	Unadjusted (n = 4958)		Adjusted[1] (n = 3800)		Adjusted[2] (n = 2696)	
	Coef (CI)	p	Coef (CI)	p	Coef (CI)	p
Never	(ref)	0.149[a]	(ref)	0.971[a]	(ref)	0.971[a]
<Once a week	-0.16 (-0.37, 0.04)		-0.017 (-0.41, 0.07)		-0.22 (-0.51, 0.08)	
At least once a week	-0.29 (-0.50, - 0.08)		-0.16 (-0.40, 0.08)		-0.21 (-0.50, 0.09)	
1-2 units every day	-0.21 (-0.44, 0.02)		-0.16 (-0.42, 0.10)		-0.27 (-0.59, 0.51)	
At least 3 glasses pd	-0.30 (-0.72, 0.13)		-0.47 (-0.95, 0.002)		-0.42 (-0.99, 0.14)	
Linear trend	-0.06 (-0.12, -0.006)	0.032	0.98 (0.91, 1.06)	0.587	-0.06 (-0.14, 0.02)	0.168

[1] Adjusted for: socioeconomic position, income, home ownership, marital status, maternal education, sex, parity, maternal tobacco use during 1-3 months of pregnancy, maternal illicit drug use during 1-3 months of pregnancy, maternal depression 18 weeks gestation

[2]Adjusted for: socioeconomic position, income, home ownership, marital status, maternal education, sex, parity, maternal tobacco use during 1-3 months of pregnancy, maternal illicit drug use during 1-3 months of pregnancy, maternal depression 18 weeks gestation, maternal PRS for major depressive disorder.

[b]Omnibus p-value

There was a negative association between maternal consumption of any alcohol when offspring were 5 years old and offspring hyperactivity at age 17 (unadjusted coefficient -0.07, 95% CI -0.14 to -0.01). Negative associations were shown for all amounts of alcohol mothers consumed (Table 4.4). After adjustment for socioeconomic factors, maternal behaviours and maternal PRS for MDD there was no clear evidence for an association (fully adjusted coefficient -0.002, 95% CI -0.09 to 0.90).

There was a negative association between maternal consumption of any alcohol when offspring were 5 years old and offspring emotional problems at age 17 (unadjusted coefficient -0.06, 95% CI -0.12 to -0.006). After adjustment for socioeconomic factors, maternal behaviours and maternal PRS for MDD there was no clear evidence for an association (fully adjusted coefficient -0.06, 95% CI -0.14 to 0.02) (Table 4.5).

There was no clear evidence that maternal alcohol amount was associated with offspring depression at age 18 or conduct problems at age 17 (Appendices 4.1 to 4.2).

Partners alcohol frequency

For partners there was a negative association between partners' alcohol consumption when offspring were 5 years and offspring total problems at age 17 (unadjusted coefficient -0.26, 95% CI -0.44 to -0.08). After adjustment for socioeconomic factors, maternal behaviours and maternal PRS for MDD there was no clear evidence for an association (fully adjusted coefficient -0.08, 95% CI -0.31 to 0.16) (Table 4.3). There was a weak negative association between partners' consumption of alcohol when offspring were 5 years old and offspring conduct problems at age 17 (unadjusted coefficient -0.05, 95% CI -0.10 to -0.0002). After full adjustment there was no clear evidence for an association (fully adjusted coefficient 0.007, 95% CI -0.06 to 0.08) (see Appendix 4.2).

There was no clear evidence that frequency of partner alcohol use when offspring were 5 years old was associated with offspring depression or hyperactivity at age 18 (see Appendices 4.1 to 4.3).

Table 4.6: Associations between partner binge drinking and offspring emotional problems (SDQ) at age 17 years

	Unadjusted (n = 2907)		Adjusted[1] (n = 2323)		Adjusted[2] (n = 1692)	
	Coef (CI)	p	Coef (CI)	p	Coef (CI)	p
None	(ref)	0.009[a]	(ref)	0.064[a]	(ref)	0.060[a]
1-2 days	-0.10 (-0.33, 0.12)		-0.15 (-0.39, 0.09)		-0.11 (-0.39, 0.17)	
3-4 days	-0.31 (-0.53, -0.09)		-0.30 (-0.55, -0.06)		-0.36 (-0.64, -0.08)	
5-10 days	-0.33 (-0.54, -0.12)		-0.31 (-0.54, -0.08)		-0.31 (-0.58, -0.04)	
>10 days	-0.24 (-0.46, -0.02)		-0.20 (-0.44, 0.04)		-0.19 (-0.47, 0.09)	
Linear trend	-0.07 (-0.12, -0.02)	0.005	0.96 (0.90, 1.02)	0.167	-0.05 (-0.11, 0.008)	0.087

[1] Adjusted for: socioeconomic position, income, home ownership, marital status, maternal education, sex, parity, maternal tobacco use during 1-3 months of pregnancy, maternal illicit drug use during 1-3 months of pregnancy, maternal depression 18 weeks gestation

[2]Adjusted for: socioeconomic position, income, home ownership, marital status, maternal education, sex, parity, maternal tobacco use during 1-3 months of pregnancy, maternal illicit drug use during 1-3 months of pregnancy, maternal depression 18 weeks gestation, maternal PRS for major depressive disorder

[a]Omnibus p-value

4.3.3 Pattern of alcohol use

There was a negative association between partners' consumption of 4 or more units of alcohol (binge drinking) when their offspring were 5 years old and offspring emotional problems (unadjusted coefficient -0.07, 95% CI -0.12 to -0.02). After full adjustment these associations were attenuated (fully adjusted coefficient -0.05, 95% CI -0.11 to 0.01) (See Table 4.6). There was no clear evidence of an association between mothers' binge drinking and offspring emotional problems at age 17 (Appendix 4.8).

There was no clear evidence that maternal or partner binge drinking when offspring were 5 years old was associated with offspring depression, conduct problems, hyperactivity or total problem scores. (see Appendices 4.4 to 4.7).

4.3.4 Confounding structures

As expected, all associations between parental drinking at age 5 and offspring mental health problems were removed after adjustment for potential confounding factors. Yet 'protective effects' were shown for increased alcohol consumption and certain mental health outcomes, and therefore mother and partner drinking patterns and confounding structures were explored as shown in Figures 4.2 and 4.3 and Table 4.2. Mothers from lower SES groups drank alcohol less frequently compared to mothers from higher SES groups. Mothers within the two lowest categories of alcohol frequency (none, 1-2 days) were more likely to be within the lower income categories, and those from the remaining three higher amounts of alcohol frequency were more likely to be within the higher income categories. The pattern of alcohol consumed (binge drinking) was fairly stable across SES.

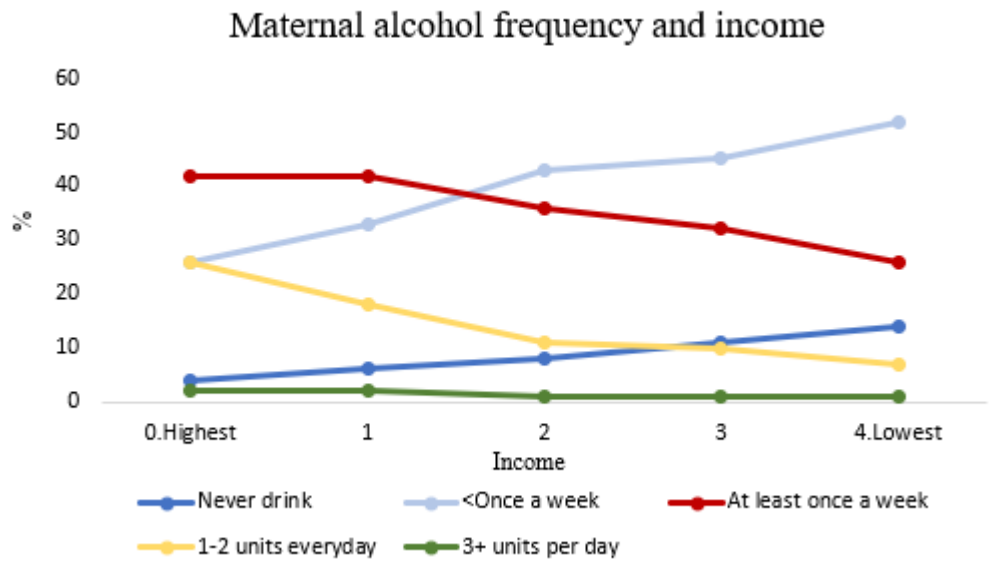

Figure 4.2: Proportion of mothers drinking frequency within income

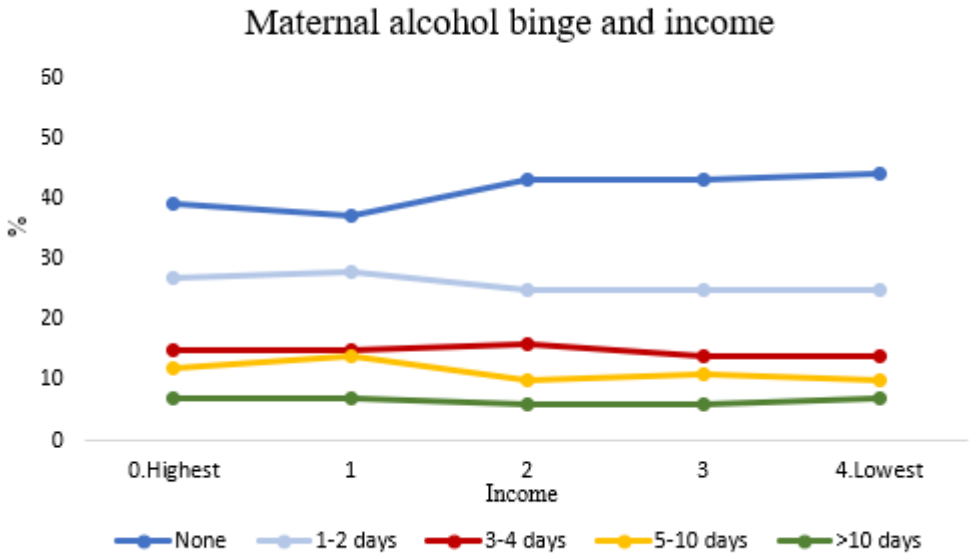

Figure 4.3: Proportion of mothers alcohol binge drinking patterns within income

4.4 Discussion

In this chapter I investigated the associations between both maternal and partner frequency and pattern of postnatal alcohol use, and offspring mental health. There was no clear evidence that parental alcohol amount or frequency was associated with offspring depression at age 18. In univariable analyses, the results suggested that increased frequency of alcohol consumption by mothers and partners was associated with reduced offspring total problems. For the subscales of mental health as measured by the SDQ, maternal alcohol amount was associated with decreased offspring hyperactivity and emotional problems at age 17. Higher frequency of partner alcohol consumption was also associated with decreased conduct problems. Parental binge drinking was not associated with any of the offspring mental health outcomes in univariate analyses, except for paternal binge drinking being associated with decreased emotional symptoms of the SDQ. However, none of these given associations remained after adjustment for socioeconomic and behavioural confounders and maternal PRS for depression. The results suggest that parental postnatal alcohol use is not associated with offspring mental health outcomes, but the findings are due to confounding structures as shown by much weaker associations from the adjustments made within adjusted models two and three for mental health outcomes.

Univariable analyses showed negative associations (increased alcohol use being associated with decreased mental health outcomes), which is in contrast to the few studies that have previously examined the association between postnatal parental drinking and offspring mental health problems (Hussong et al., 2008; Hussong et al., 2010). However, this could be because this chapter focused mainly on light to moderate alcohol consumption and previous studies often investigated extremes of alcohol use and parents with AUD. The current study did not however find associations between parental alcohol use and offspring mental health problems in multivariate analyses, indicating that any associations shown within the unadjusted models was due to confounding factors.

To further test what these findings may be reflecting, I sought to establish what the differences seen in the univariate analyses between parental alcohol amount and pattern (binge drinking) were related to. Differences were shown between levels of alcohol frequency and pattern between socioeconomic factors. Mothers who reported never drinking alcohol and drinking alcohol less than once a week were more likely to be from lower socioeconomic backgrounds. Mothers from higher socioeconomic backgrounds showed higher amounts of drinking at least once a week and 1-2 units everyday compared to mothers from lower socioeconomic backgrounds. This indicates that of the 5 possible responses to amount of alcohol consumed, the two lowest amounts

were more likely to be reported by those with lower SES, and the three higher categories of alcohol amount were reported more by those with higher SES. This suggests that the apparent 'protective effect' shown from greater alcohol consumption being associated with reduced mental health problems, is likely to in fact be due to differences in the confounding structures within the data, and that factors such as socioeconomic status and income are what is actually protective against mental health problems. This is further demonstrated by the findings of no clear evidence of an association between parental binge drinking and offspring mental health problems in the univariate analyses. Further investigation of parental binge drinking patterns and socioeconomic status showed more comparable amounts of binge drinking between varying levels of income, which is likely why the same findings are not shown for binge drinking and offspring mental health. The associations of alcohol amount therefore seem to be heavily influenced by confounding factors such as socio-economic status. This is in line with previous research which has shown a J-shaped curve in alcohol research and negative health outcomes. The J-shaped curve suggests there are potential health benefits of consuming moderate amounts of alcohol (Chokshi, El-Sayed, & Stine, 2015; Di Castelnuovo et al., 2006) compared to abstinence from alcohol. This is argued to be due to the reasons often attributed to people abstaining from alcohol use, such as ill health. Therefore, the increased risk often seen for poor health for abstainers may instead be due to former drinkers being a part of the abstinence group. The J-shaped curve highlights the necessity for statistical adjustment of potential confounders.

There are limitations to the current study that should be considered. Self-reported alcohol use is often under-reported which may have biased our findings, by weakening any associations shown. However, it has been previously suggested that reports outside of pregnancy are often more reliable compared to reports given about alcohol consumption during pregnancy (Alvik et al., 2006), which could have influenced mothers accurate reports through fear of being stigmatised. Secondly, partner alcohol use was not self-reported but instead given by mothers who were asked to report on their partners consumption. Using self-report data would have been optimal, but due to the much-reduced sample size of available data from partners on their own alcohol use, this was not possible due to the rate of attrition. The sample size for both exposures of maternal and paternal alcohol use and offspring outcomes were small, and could therefore have been underpowered to detect a true association.

In previous chapters I have shown that maternal alcohol use in pregnancy is associated with increased offspring depression, but the results reported in the current chapter suggest that postnatal alcohol use is not associated with offspring mental health problems and associations shown in the unadjusted models were due to confounding

structures. The exposure and outcome measures used so far can suffer from problems of bias in misreporting, particularly for alcohol exposure during pregnancy, we could therefore look for other methods which may be slightly more representative such as genetic markers of alcohol use. In trying to keep the research questions refined I have also somewhat used limited mental health outcome measures. This means that I may be missing pathways to other types of mental health outcomes which were not measured in the current study which focused on the SDQ subscales and depression only. Future work should find ways to include other relevant mental health outcomes that may be influenced by maternal alcohol consumption, as well as at different offspring ages to help discover if there are sensitive timepoints for risk.

4.5 Chapter summary

In this chapter I investigated the associations between both maternal and partner postnatal alcohol use (when offspring were 5 years old) and offspring mental health problems. Univariate analyses suggested that increased parental alcohol amount was associated with decreased mental health problems. However, further investigation and adjustment for potential confounders indicated that the previously seen associations were due to differences in confounding structures within the amount of alcohol consumed. The findings from this chapter therefore indicate that postnatal alcohol use, particularly at low to moderate levels, is not associated with offspring mental health problems. As Chapter Three suggested that intrauterine alcohol exposure was associated with offspring depression, the following chapter will investigate other mental health outcomes that prenatal alcohol use may be negatively influencing. However, this shall now be conducted using PRS for alcohol use to investigate potential pathways of risk.

Chapter 5 The association of alcohol polygenic risk scores on mental health phenotypes: A PheWAS in the Avon Longitudinal Study of Parents and children

In Chapter 4 I investigated the associations between parental postnatal alcohol use and offspring mental health problems. I found increased parental alcohol intake to be associated with positive offspring mental health. However, further investigation suggested these associations were a result of confounding structures within the data and increased parental postnatal alcohol use was not actually providing a protective effect against mental health problems. The following chapter aims to build on my previous chapters, which showed associations between maternal prenatal alcohol exposure and offspring mental health, by investigating the effects of genetic variants for alcohol use (both maternal and child's own) on a wide variety of mental health outcomes using an emerging technique of a Phenome Wide Association Study (PheWAS). By using genetic variants for increased alcohol consumption I have created a better indicator of alcohol use for the exposure, which may be more reliable than self-reported alcohol use in pregnancy.

5.1 Introduction

Genome Wide Association Studies (GWAS) are used to test associations between traits (often diseases) and common SNPs, to allow the identification of common risk variants to a single trait. Although extremely successful in previously identifying risk variants for disease (Manolio et al., 2009; Visscher et al., 2017), this technique typically focuses on a single pre-determined trait of interest and may therefore miss other key phenotypic associations.

An emerging technique is that of a PheWAS, which essentially reverses the phenotype to genotype methods used in a GWAS, to become a genotype to multiple phenotype approach (Figure 5.1). Comparable to a GWAS, this type of analysis is still hypothesis free, but this time for the outcomes instead of the exposures. A PheWAS takes a pre-determined set of genetic variants shown through a GWAS to affect a specific trait, and tests which of a wide range of phenotypes these genetic variants of interest may be associated with. Due to increasing phenotypically rich datasets and the growth in available genotypic data, a PheWAS can identify new SNP to disease associations. By investigating multiple phenotypes using a PheWAS, we can investigate cross-phenotype

associations within the same study population (Namjou et al., 2014; Pendergrass et al., 2013). Population cohorts, such as ALSPAC are a useful source of rich phenotypic data with outcomes not only for children within the study, but the pregnant mothers also. We can further investigate the genetic architecture of multiple traits and disease outcomes through linking a chosen genetic variant to multiple phenotypes, in varying subpopulations.

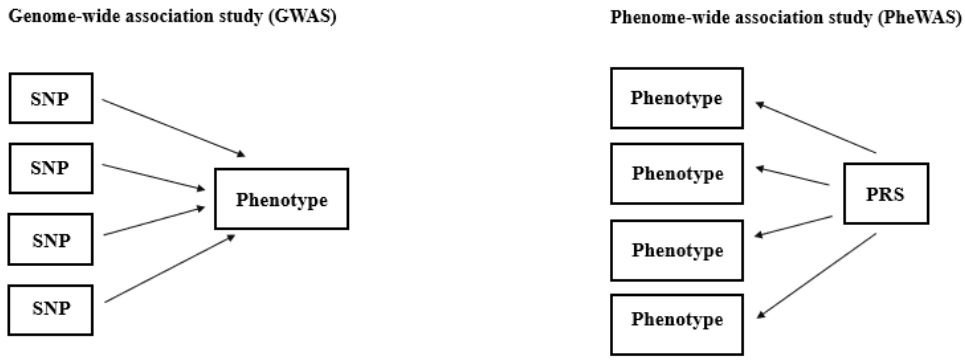

Figure 5.1: Differences between GWAS and PheWAS. In a GWAS, the whole genome is regressed on a single trait. In a PheWAS, the phenome is regressed on a single or small number of genetic variants.

Many PheWASs utilise electronic medical records which form a large phenotypic database, to test for associations with predefined genetic variants. We are unable to truly measure the entire phenome, and therefore the strength of using a PheWAS design is shown within the phenotypes that are available within each dataset used. There are two main methods for conducting a PheWAS, a non-targeted and a targeted approach (Barnado et al., 2018; Diogo et al., 2018; Millard et al., 2015; Verma et al., 2019). The first, is to investigate the effect of genetic variants on all available phenotypes within a study, in a non-targeted approach. The second, is to investigate the effect of genetic variants on a specific set of phenotypes (e.g., all phenotypes related to blood pressure), in a targeted approach. This approach is beneficial in allowing a more refined research question. The current chapter used a targeted PheWAS, which measured the available and pertinent phenotypes representing mental health constructs within ALSPAC. Previous PheWASs have validated the method, as shown by the first published PheWAS which investigated genetic variants across a set of phenotypes in a targeted approach (Denny et al., 2010); this study provided a proof of principle.

The benefits of conducting a PheWAS are shown in the main inferences we can conclude from their results. The first is in understanding pleiotropic effects, which is where a single genetic variant influences multiple traits (Paaby & Rockman, 2013), this can take two forms; vertical and horizontal pleiotropy (see Figure 5.2). Vertical pleiotropy is where a genetic variant influences one trait which then in turn influences another, akin to mediation analyses. Horizontal pleiotropy occurs when a genetic variant influences multiple causal pathways, such as another trait which then influences the outcome independently of the exposure (Burgess, Foley, & Zuber, 2018). Horizontal pleiotropy is a limitation of such studies and can result in biased estimates.

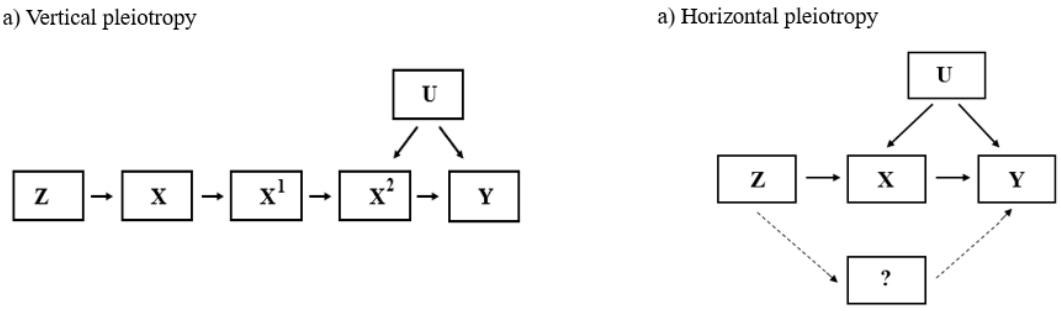

Figure 5.2: Differences between horizontal and vertical pleiotropy. *Vertical pleiotropy*: Genetic instrument (Z), for the exposure of interest (X), is associated with (Y) via other traits (X_1, X_2) on the **causal/same** pathway. *Horizontal pleiotropy:* Genetic instrument (Z), for the exposure of interest (X) is associated with the outcome (Y), through another trait (?) on an alternative pathway, instead of or in addition to the exposure of interest (X)

A PheWAS aids in investigating genetic variants which may have pleiotropic effects and affect more than one phenotypic trait, it can therefore inform our understanding of the pleiotropic effects of genetic variants. The second main inference we can make from a PheWAS is the potential causal pathways from genetic variants to mental health problems. If genetic variants for increased alcohol use are shown to affect these mental health phenotypes, these variants can therefore be a risk pathway for mental health problems.

Genetic variants have been associated with increased alcohol consumption before pregnancy, but less research has been published for their influence during pregnancy. A variant within the alcohol dehydrogenase ADH_1B gene is involved in metabolizing ethanol and has been linked with alcohol dependence. Zuccolo and colleagues (Zuccolo et

al., 2009) further investigated the role of these variants on alcohol use in pregnancy, compared to invitro effects which had previously only been studied after pregnancy. Zuccolo and colleagues confirmed the ADH_1B gene was associated with a lower alcohol consumption before and during pregnancy. Variants within the ADH_1B gene may protect against adverse outcomes from alcohol exposure, however, the amount of variance accounted for is small (Dodge, Jacobson, & Jacobson, 2014; Jacobson et al., 2006). The use of a candidate gene such as this is hypothesis driven and assumes knowledge about the underlying functional pathways and biology. In recent years more genomic data has become available at an affordable cost, meaning we are now able to scan the entire genome for genetic variation of polymorphisms. From this, SNPs that have been shown to be robustly associated with a trait of interest can be combined to create PRS. These PRS can then be used as instrumental variables for alcohol use within analyses. Pregnant mothers can therefore influence their offspring through both the intrauterine environment and their genetic liability.

Alcohol use is known to be comorbid with mental health problems (Jané-Llopis & Matytsina, 2006), however, we do not yet fully understand all mental health subtypes that may be associated with alcohol consumption, and particularly which mental health problems are also associated with genetic variants for alcohol use. To be able to investigate this, one approach is to first establish strong causal variants related to increased alcohol use. PRS are created by collating multiple genetic markers from a large-scale association study, that on their own may not be significant, into a score that predicts risk of disease (Dudbridge, 2013). A recent study of approximately 1.2 million participants, discovered 99 genetic variants associated with increased alcohol use (Liu et al., 2019). By using such a large sample size of multiple cohorts, this study provides a much better measure to further investigate the effects of substance use measures. PRS provide greater statistical power to identify evidence for associated loci, achieved mostly through very large increases in sample sizes (Duncan, Ostacher, & Ballon, 2019). However, PRS still explain a small amount of phenotypic variance.

In this study I constructed PRS from the SNPs identified by Lui and colleagues which were shown to be robustly related to alcohol use (Liu et al., 2019), to use in a PheWAS approach. My objectives were:

1. To validate these genetic signals within the association of alcohol phenotypes in pregnant women, and two sub populations of offspring (age ~7 and ~18). I expect weaker effects for offspring phonotypes. This is because offspring would have 50% shared genetic data with their mother, and I would therefore expect the effects found to be roughly halved compared to the mother's analyses.

2. To test if there are any associations (other than with alcohol use) of these PRS with a large number of mental health phenotypes. This will be tested through a targeted PheWAS approach within subpopulations of:
    a. Mothers PRS and mothers' mental health phenotypes.
    b. Offspring PRS and offspring mental health phenotypes at age ~18. This was conducted as close to age 18 as available phenotypes allowed.
    c. Offspring PRS and offspring mental health phenotypes at age 7. This was conducted as close to age 7 as available phenotypes allowed. This aim was included as a negative control design, as children are likely to have not started consuming alcohol at this age. This would indicate if any associations between PRS and mental health phenotypes were due to alcohol use itself, or independent of it and therefore pleiotropic with mental health traits.
3. Lastly, maternal PRS for alcohol use will be tested for associations with offspring mental health and alcohol phenotypes at the same two ages as objectives 2b and 2c to investigate maternal intergenerational effects.

5.2 Methods

5.2.1 Study population

The ALSPAC cohort as described in Chapter Three was again used within the current chapter. Pregnancies were excluded if they were triplets or quadruplets, and siblings were also removed from the analyses.

5.2.2 Genotyping and quality control

Children from the ALSPAC cohort were genotyped using the Illumina HumanHap550 quad chip genotyping platforms by 23andme subcontracting the Wellcome Trust Sanger Institute, Cambridge, UK and the Laboratory Corporation of America, Burlington, NC, US. Mothers from ALSPAC were genotyped using the Illumina human660W-quad array at Centre National de G*énotypage* (CNG) and genotypes were called with Illumina GenomeStudio. PLINK (v1.07) was used to carry out quality control measures on an initial set of 10,015 subjects and 557,124 directly genotyped SNPs. Mothers SNPs were removed if they displayed more than 5% missingness or a Hardy-Weinberg equilibrium P value of <1.0e-06. Population stratification was assessed by multidimensional scaling analysis and compared with Hapmap II (release 22) European descent (CEU), Han Chinese, Japanese and Yoruba reference populations; all individuals with non-European ancestry were removed. This

then combined 477,482 SNP genotypes in common between the sample of mothers and sample of children. Additionally, if any SNPs had a minor allele frequency of <1% or had genotype missingness >1% because of poor genotyping, they were also removed (11,396 SNPs removed). A further 321 subjects were removed because of potential ID mismatches. After this genotyping and quality control, a dataset of 17,842 subjects remained. This contained 6,305 duos and 465,740 SNPs. After related subjects were excluded, 8,237 eligible children and 8,196 eligible mothers with available genotype data remained. Further detail of the quality control process is described in (Paternoster et al., 2011).

5.2.3 Alcohol polygenic risk scores

A recent large scale GWAS (Liu et al., 2019) used over 1.2 million individuals and identified 99 SNPs related to the number of alcoholic drinks consumed per week. Within their analyses multiple studies (up to 34) were used, including UK Biobank, 23andMe and ALSPAC. Lui and colleagues defined drinks per week as the average number of alcoholic drinks (aggregated across all alcohol types) consumed per week. Due to multiple studies included within their analyses, varying measures of alcohol consumption were recorded. If included studies used categorical responses (e.g., 1-5 drinks per week) the midpoint was used (e.g., 1-5 drinks per week was recorded as 2.5 drinks per week). To stop potential outliers leveraging any analyses, the drinks per week phenotype was left-anchored at 1 and log-transformed by Lui and colleagues prior to analyses. I calculated PRS for alcohol use based on these genome-wide significant SNPs (see Appendices 5.1) associated with the amount of alcoholic drinks per week, weighted by the effect estimates reported by Lui and colleagues for the full sample included within their study. The PRS for alcohol use were calculated both for the mothers and their offspring, PLINK V1.9 was used for this computation.

5.2.4 Phenotyping

Targeted phenotypes were selected from available substance use (*n* = 22*)* and mental health/behavioural variables (n = 90) within ALSPAC. In the mother's phenotypes (*n* = 29; see Figure 5.3), variables were recorded during pregnancy (8 to 32 weeks gestation). In the child's phenotypes (*n* = 61; see Figure 5.4), variables were recorded as close to age 18 where phenotypically possible. Variables measuring the same outcome were also recorded as close to age 7 as possible, before alcohol initiation was likely to have occurred. Where multiple measures were available which measured the same

construct or the same underlying phenotype at the same age, each were tested for correlation. If variables were found to be highly correlated, the variable with the biggest sample size was selected to maximise sample size. Continuous variables were favoured over binary variables of the same measure.

5.2.5 Mothers' phenotypes

All maternal phenotypes (see Figure 5.3) were self-reported. Measures of maternal alcohol use during pregnancy were continuous measures of, alcohol consumption in months 1-3 of pregnancy, number of days mothers binge drank (4+ units of alcohol in the past month) at 18 and 32 weeks gestation, total weekly units of alcohol consumed at 32 weeks gestation. Binary measures were, the most consumed alcoholic beverage (compared to other alcoholic drinks during pregnancy) at 18 weeks gestation, being wine, beer or lager, spirits, sherry or port, other types of alcohol. As well as measures indicating change in alcohol consumption during pregnancy or not (increased use/stopped use/no change/increased craving/never used), for mothers who normally consumed alcohol and those who normally did not consume alcoholic drinks.

Continuous measures of maternal mental health during pregnancy at 18 weeks gestation were neurotic symptomatology (Crown Crisp Experiential Index), hypersensitivity to personal rejection, image perception within the last four weeks, image perception change from 3 months pre-pregnancy to 18 weeks gestation and reactions towards becoming a parent.

Continuous measures at 32 weeks gestation were total caffeine consumption, highest maternal education qualification, and mother's perception of their own physical activity compared to other pregnant women of a similar age.

Separate binary measures at 8 weeks gestation measured changes (increased use/stopped use/no change/increased craving/never used) of caffeine consumption during pregnancy, as well as tobacco use. Binary phenotypes measured at 18 weeks gestation were included of if mothers had illicit drug use, smoked during pregnancy, taken cannabis during pregnancy, vomited during pregnancy and if mothers had ever smoked in their lifetime. Depression measures at both 18 and 32 weeks gestation were included based on the Edinburgh Postnatal Depression Scale (Cox et al., 1996), at both 18 and 32 weeks gestation, with scores ≥13 indicating a diagnosis of depression.

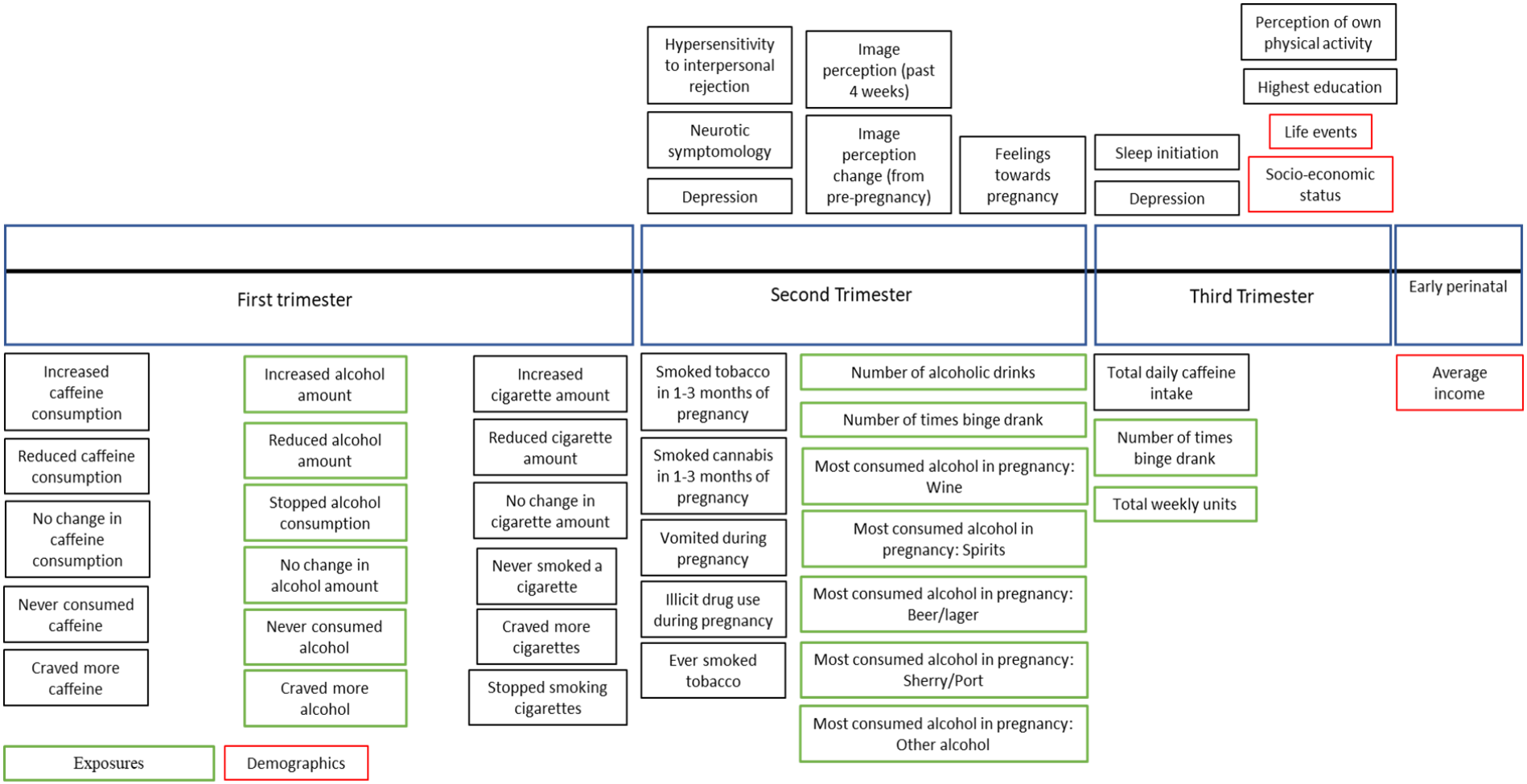

Figure 5.3: Phenotypes included in PheWAS for mothers during pregnancy

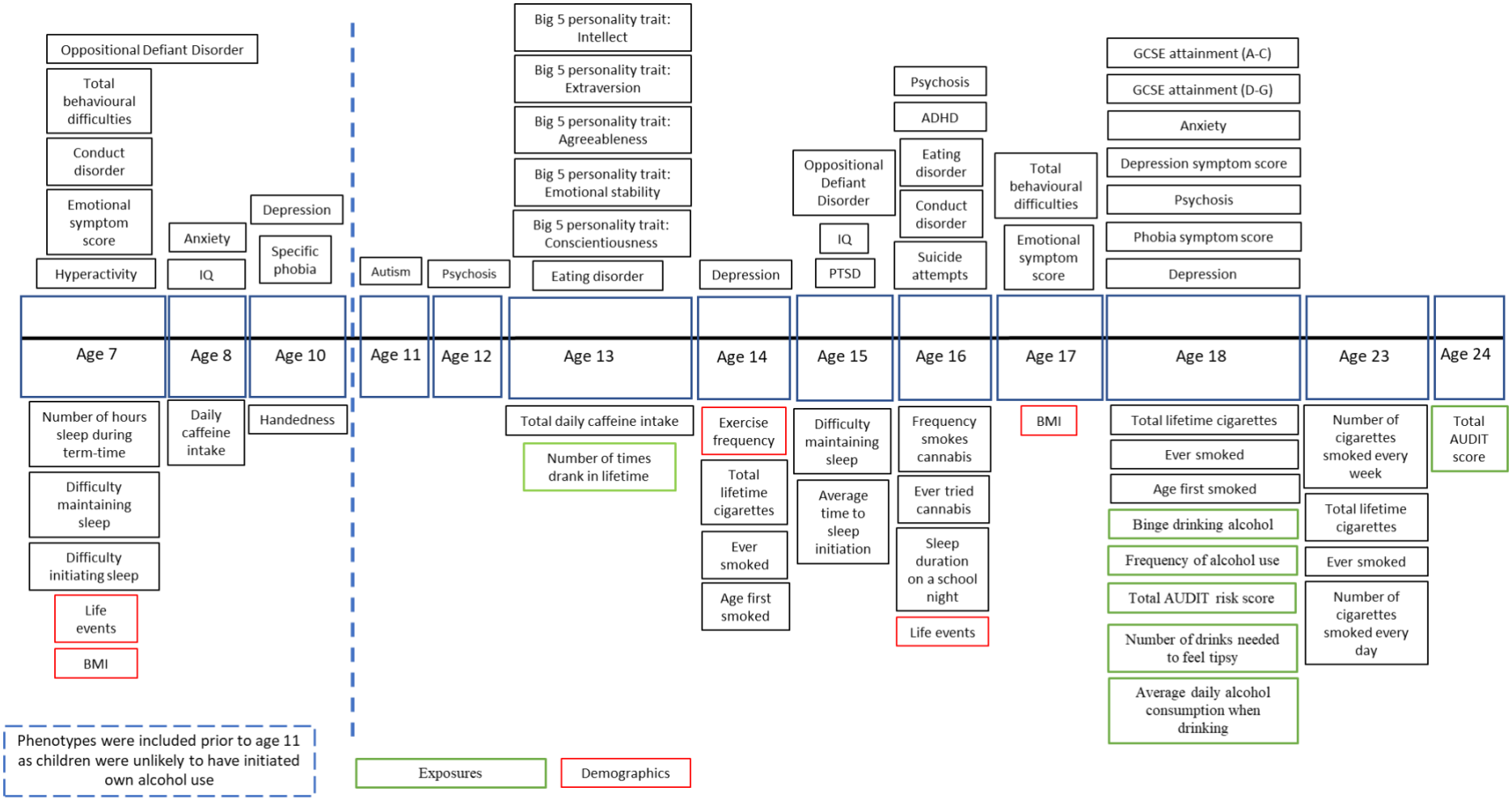

Figure 5.4: Phenotypes included in PheWAS for child and adolescents

Maternal covariates of interest were included of continuous measures of maternal socioeconomic status, and number of adverse life events during pregnancy at 32 weeks gestation, and a combined measure of average income (measures at 33 and 47 months).

5.2.6 Child phenotypes

See Figure 5.4 for all child and adolescent phenotypes. Measures of adolescent alcohol use were self-reported measures at age 18 of alcohol frequency, amount of alcohol consumed daily, number of days they binge drank (4+ units of alcohol) in the past month, the number of drinks it took to feel tipsy and a total score for hazardous drinking behaviours from the Alcohol Use Disorders Identification Test (AUDIT), as well as a measure of the number of times they had consumed alcohol at age 13. A total score from the Alcohol Use Disorders Identification Test (AUDIT) was included at age 24. The AUDIT measure has been shown to be a reliable indicator of alcohol use, and the validity and reliability of this measure have been previously calculated. A review showed the internal consistency to have a mean Cronbach's alpha coefficient of 0.8, and internal consistency of 0.9 (de Meneses-Gaya et al, 2009).

Childhood continuous measures were recorded as close to age 7 as were available. These were number of hours the child normally sleeps during term-time, presence of oppositional defiant disorder (ODD), conduct disorder symptoms, hyperactivity symptoms, emotional symptoms score, and total behavioural difficulties score from the Strengths and Difficulties Questionnaire (SDQ) (Goodman, 1997; Woerner et al., 2004) at age 7. A general anxiety symptoms scale and child's IQ was also measured at age 8, and a total depression score from the Short Mood and Feelings Questionnaire (SMFQ) (Angold et al., 1995) at age 10. Daily caffeine intake was measured at age 8.

Binary measures of a clinical diagnosis of specific phobias at age 10, and measures of difficulty initiating and maintaining sleep were also included from age 7. A measure of handedness was included as a proof of principle measure (e.g. a 'negative control outcome' measure as it would not be expected to find an association between the PRS and handedness).

Child covariates of interest were a continuous measure of body mass index (BMI) and number of negative life events the child had at age 7.

Adolescent continuous measures were phobia symptoms, total anxiety scores and sum of the all the 5 depression symptom subscales from the Clinical Interview Schedule-Revised (CIS-R) (Lewis et al., 1992) at age 18, depression symptom score as well as total

score for the SMFQ measuring depression at age 18 and 14. Self-reported negative psychotic symptom scores were measured by the Community Assessment of Psychotic Experiences (CAPE) (Yung et al., 2009) at age 16, as well as a total score of emotional symptoms, total problem score as measured by SDQ at age 17. A total score of ADHD, conduct disorder and frequency of cannabis use were also measured at age 16. ODD, a clinical diagnosis of Post-Traumatic Stress disorder (PTSD) was measured by the Development and Wellbeing Assessments (DAWBA), as well as IQ and measures of initiating and difficulty maintaining sleep were measured at age 15. Average duration of sleep on a school night was measured at age 16. Daily caffeine intake was measured at age 13, as well as personality measures of agreeableness, extraversion, emotional stability, intelligence and conscientiousness. Measures of psychotic like symptoms (PLIKS) at age 18 and 12 were included, as well as tobacco measures of age first smoked measured at ages 14 and 18, total number of cigarettes smoked in lifetime at ages 18 and 23, number of cigarettes smoked per day and per week at age 23.

Adolescent binary measures were a diagnosis of autism at age 11, presence of an eating disorder at ages 13 and 16, ever self-harmed with suicidal intent at age 16, have ever smoked a cigarette by age 14, total number of cigarettes smoked at age 14, ever smoked at ages 18 and 23, if ever tried cannabis by age 16, and if obtained a GCSE grade at levels A*-C, or D-G.

Adolescent covariates of interest were continuous measures of BMI age 17, frequency of exercise at age 14 and negative life events at age 16.

5.2.7 Statistical analysis

Using Stata version 15.1, linear and logistic regression analyses were used to investigate if PRS for alcohol use were associated with 1. Dimensions of alcohol use in mothers in pregnancy, and for offspring, and 2. Mental health phenotypes (for mothers during pregnancy, and for children both pre-alcohol use around ages 7-10, and post-alcohol use around ages 13-23), and 3. Intergenerational effects were tested using maternal PRS for alcohol use with offspring phenotypes. Complete case analyses were conducted and therefore all missing observations were dropped due to missing genetic data which failed quality control. All analyses were performed separately in children and mothers. Analyses were adjusted for sex (in the children only), age (at questionnaire completion or clinic attendance), and the first 10 ancestry-informative principal components. Principal components capture geographical ancestry, and by adjusting for these we are able to adjust for small differences in population structure which can

otherwise cause spurious associations (Price et al., 2006). Principal components are calculated by partitioning the genetic variance of a population into components that explain each one. Each person has a value of how much they load on to each component, and adjustment of these removes similarities due to population structure. How many principal components are used depends on the homogeneity of the sample used, with more heterogeneous samples (more diverse ancestry) requiring a greater amount of principal components (Anderson et al., 2010).

All continuous phenotypes were checked for zero-inflated variables and normal distribution, and those that were not normally distributed were normalised by creating quantiles. Phenotypes with >30% zero-inflation were rank transformed into 3 quantiles (0, ≤ median, > median). Non-ordered categorical phenotypes were transformed into binary variables of where appropriate, indicating the presence of an outcome compared to a control. All non-binary variables were treated as continuous in linear regression analyses.

Miami plots were produced for a graphical representation of the results. Heat Maps are given to represent the relationship between effects shown between offspring and intergenerational analyses (aims 2b, 2c and aim 3). Phenotypes were included for comparison where the same measure was used across different generations. Bonferroni testing was conducted to correct for multiple testing. Due to the conservative nature of Bonferroni testing, sensitivity analyses using permutation tests were also conducted as this non-parametric test has weaker assumptions.

5.3 Results

The results include 165 tests categorised by generation tested (e.g. maternal/child/intergenerational) and ranked by p-value. Results are summarised in separate tables for each study aim. Aim 1 (validation of alcohol PRS) is shown in Table 5.2, aim 2a (mothers PRS and mother's phenotypes within pregnancy) in Table 5.3, aims 2b and 2c (offspring PRS and offspring phenotypes ages 7 and 18) in Table 5.4, and aim 3 (intergenerational, mothers PRS and offspring phenotypes ages 7 and 18) in Table 5.5.

Aim 1) Validation of PRS for alcohol use and alcohol phenotypes

Maternal PRS for alcohol use previously shown in the general population were validated for maternal alcohol use during pregnancy (Table 5.1 and Table 5.2). The strongest evidence was shown for the amount of alcohol drank ($p = 1.01 \times 10^{-5}$) at 18

weeks gestation, binge drinking at 18 ($p = 9.19 \times 10^{-4}$) and 32 weeks ($p = 2.37 \times 10^{-4}$) gestation, and weekly units at 32 weeks gestation ($p = 1.70 \times 10^{-5}$). There was no evidence that offspring PRS for increased alcohol use was associated with any of the offspring alcohol measures at either of the two measures timepoints. Maternal PRS for increased alcohol use was validated only for increased offspring AUDIT total score at age 18 (see Table 5.2).

Table 5.1: Adjusted r^2 values between own alcohol PRS and alcohol phenotypes

Mother	**Beta**	**R^2**
Alcohol amount	0.041	1.6%
Binge drinking frequency (18 weeks)	0.030	0.1%
Weekly units	0.251	1.1%
Binge drinking frequency (32 weeks)	0.036	0.5%
Child		
Alcohol frequency	0.024	2.8%
Audit risk score	0.016	0.9%
Binge drinking frequency	0.010	2.4%
Number of drinks to feel tipsy	-0.024	4.2%
Average drink total per day	-0.004	0.5%
Number of times had whole drink	0.346	0.06%
AUDIT total score	0.127	3.0%

Aim 2a) Maternal PRS for alcohol use and mother's mental health phenotypes within pregnancy

Maternal PRS for alcohol use was associated with increased maternal depression at 32 weeks gestation (OR = 1.09, 95% CI = 1.02-1.18), see Table 5.3. Figure 5.5 indicates the strength of evidence for maternal PRS for alcohol and maternal phenotypes during pregnancy.

Aims 2b and 2c) Offspring PRS for alcohol use and offspring phenotypes (age ~7 and age ~ 18)

There was no evidence that offspring PRS for alcohol use were associated with any of the adolescent alcohol measures, or any of the child mental health phenotypes, see Tables 5.2 and 5.4.

Aim 3) Intergenerational: Maternal PRS for alcohol use and offspring phenotypes (age ~7 and age ~ 18)

Maternal PRS for alcohol use was associated with increased offspring AUDIT total score (Coef = 0.184, 95% CI = 0.02, 0.35, $p = 0.028$). Of the measured offspring phenotypes, maternal PRS for alcohol use was associated with decreased scores in intellectual ability at age 13 (Coef = -0.209, 95% CI = -0.38, -0.04), see Tables 5.2 and 5.5.

Heatmaps are given for comparisons between subpopulations of child PRS and child phenotypes, and maternal PRS and child phenotypes (intergenerational) demonstrating the size of effects (see Figures 5.8 and 5.9). Miami plots have also been produced to further illustrate the strength of associations between PRS for alcohol use and phenotypes within each subpopulation (see Figures 5.5 to 5.7).

Bonferroni correction used an α value of 0.05 / number of tests within each analysis (maternal PRS and maternal outcomes: 29; offspring PRS and offspring outcomes age 7: 16; offspring PRS and offspring outcomes age 18: 45; intergenerational age 7: 16; intergenerational age 18: 45). After Bonferroni correction (see Appendices) the strength of evidence became weak for any of the mental health phenotypes, in any of the subpopulation analyses (maternal Bonferroni threshold: $p = 0.002$; child and intergenerational Bonferroni threshold: $p = 0.0008$). Permutation analyses were conducted and showed the strength of evidence persisted for subpopulations of maternal PRS on maternal phenotypes (maternal depression at 32 weeks gestation: $p = 0.016$). No effects were again shown for child PRS on child phenotypes. Within the intergenerational subpopulation of maternal PRS on offspring phenotypes, the strength of evidence that was originally shown for intellectual ability at age 13 no longer persisted.

Table 5.2 Associations between alcohol PRS and alcohol phenotypes for mothers and offspring

Exposure	Age	Effect Size	OR/beta	lowerCI	upperCI	pvalue	*n*
Maternal outcomes							
Alcohol amount	18 weeks gestation	beta	0.041	0.02	0.06	1.01×10^{-5}	7185
Weekly units	32 weeks gestation	beta	0.251	0.14	0.36	1.70×10^{-5}	4294
Binge drinking frequency	32 weeks gestation	beta	0.036	0.02	0.06	2.37×10^{-4}	5324
Binge drinking frequency	18 weeks gestation	beta	0.030	0.01	0.05	0.001	7171
Most consumed alcoholic drink: Wine	18 weeks gestation	OR	1.109	1.04	1.18	0.004	5199
Reduced alcohol amount	8 weeks gestation	OR	1.077	1.02	1.14	0.011	6771
Never drinker	8 weeks gestation	OR	0.925	0.87	0.98	0.017	6771
Most consumed alcoholic drink: Beer/lager	18 weeks gestation	OR	1.101	1.02	1.19	0.021	3667
No change in alcohol amount	8 weeks gestation	OR	0.930	0.85	1.02	0.101	6771
Most consumed alcoholic drink: Other alcohol	18 weeks gestation	OR	1.065	0.96	1.18	0.212	3054
Craved more alcohol	8 weeks gestation	OR	0.760	0.46	1.27	0.264	6771
Stopped drinking alcohol	8 weeks gestation	OR	1.025	0.96	1.09	0.424	6771
Most consumed alcoholic drink: Spirits	18 weeks gestation	OR	1.065	0.83	1.37	0.593	2582
Most consumed alcoholic drink: Sherry/port	18 weeks gestation	OR	1.048	0.79	1.39	0.723	2540
Child outcomes							
Alcohol frequency	18	beta	0.024	-0.01	0.05	0.116	2886
AUDIT total score	24	beta	0.127	-0.03	0.29	0.121	2696

AUDIT risk score	18	beta	0.016	-0.01	0.04	0.188	3008
Number of times had whole alcoholic drink	13	beta	0.346	-0.18	0.87	0.198	1103
Binge drinking frequency	18	beta	0.010	-0.04	0.06	0.675	2829
Number of drinks to feel tipsy	18	beta	-0.025	-0.15	0.10	0.698	2391
Average drink total per day	18	beta	-0.004	-0.05	0.04	0.859	2826
Intergenerational outcomes							
AUDIT total score	18	beta	0.184	0.02	0.35	0.028	2516
Number of times had whole alcoholic drink	18	beta	0.437	-0.07	0.94	0.090	1012
Average drink total per day	18	beta	-0.023	-0.07	0.02	0.326	2647
Alcohol frequency	24	beta	0.012	-0.02	0.04	0.440	2702
Binge drinking frequency	18	beta	0.013	-0.03	0.06	0.594	2651
AUDIT risk score	18	beta	0.006	-0.02	0.03	0.630	2812
Number of drinks to feel tipsy	18	beta	-0.005	-0.12	0.11	0.932	2246

Table 5.3: Associations between maternal alcohol PRS and maternal mental health phenotypes

Phenotype	Age	Type	OR/beta	lowerCI	upperCI	pvalue	*n*
Depression	32 weeks	OR	1.097	1.02	1.18	0.022	6751
Neuroticism	18 weeks	beta	0.164	-0.02	0.35	0.078	6456
Life events	32 weeks	beta	0.013	0.00	0.03	0.084	6936
Education	32 weeks	beta	0.024	0.00	0.05	0.102	6956
Smoked 1-3 months	18 weeks	OR	1.049	0.98	1.12	0.126	7237
Depression	18 weeks	OR	1.063	0.98	1.15	0.131	6734
Ever smoked	8 weeks	OR	0.958	0.90	1.02	0.152	6719
Social class	32 weeks	beta	-0.019	-0.05	0.01	0.164	5854
Reduced cigarettes	8 weeks	OR	1.051	0.98	1.13	0.174	6719
Increased cigarettes	8 weeks	OR	1.421	0.79	2.54	0.213	6719
Smoked cannabis 1-3 months	18 weeks	OR	1.104	0.94	1.30	0.214	6918
Vomited in pregnancy	18 weeks	OR	0.969	0.92	1.02	0.225	6797
Daily caffeine intake	8 weeks	beta	1.408	-1.16	3.98	0.283	6769
Hypersensitivity to rejection	18 weeks	beta	0.177	-0.20	0.55	0.351	7169
Sleep initiation	32 weeks	beta	0.008	-0.01	0.03	0.417	6745
No change in caffeine	8 weeks	OR	0.980	0.93	1.03	0.431	7269
Image perception	18 weeks	beta	0.036	-0.06	0.13	0.474	6701
Increased caffeine	8 weeks	OR	0.971	0.89	1.07	0.510	7269

Physical activity perception	32 weeks	beta	0.006	-0.01	0.02	0.515	6716
Craved more caffeine	8 weeks	OR	1.023	0.94	1.11	0.552	7269
Ever drank caffeine	8 weeks	OR	0.987	0.94	1.04	0.585	7269
Reduced caffeine	8 weeks	OR	0.990	0.94	1.04	0.675	7269
No change in cigarettes	8 weeks	OR	1.017	0.90	1.15	0.783	6719
Image perception change	18 weeks	beta	-0.010	-0.10	0.08	0.824	6551
Stopped smoking	8 weeks	OR	1.010	0.91	1.12	0.830	6719
Illicit drugs in pregnancy	18 weeks	OR	0.957	0.62	1.48	0.830	7147
Reaction to becoming a parent	18 weeks	beta	-0.002	-0.02	0.02	0.831	7167
Craved more cigarettes	8 weeks	OR	1.020	0.65	1.61	0.926	6719
Average income	33/47 months	beta	0.000	-0.03	0.03	0.983	5430

Table 5.4: Associations between offspring alcohol PRS and offspring mental health phenotypes

Phenotype	Age	Type	OR/beta	lowerCI	upperCI	pvalue	*n*
Children							
Handedness	10	OR	0.922	0.84	1.01	0.071	5399
Sleep duration	7	beta	-0.020	-0.04	0.00	0.087	5445
Sleep maintenance	7	OR	0.954	0.90	1.02	0.139	5451
Conduct disorder	7	beta	0.003	-0.02	0.02	0.767	5329
Specific phobia	10	OR	1.142	0.82	1.59	0.402	5473
Life events	7	beta	0.008	-0.01	0.03	0.405	5496
Daily caffeine intake	8	beta	0.268	-0.56	1.09	0.525	4589
Oppositional defiant disorder	7	beta	0.007	-0.01	0.03	0.526	4859
Hyperactivity symptoms	7	beta	-0.005	-0.02	0.01	0.549	5222
BMI	7	beta	-0.015	-0.07	0.04	0.582	5799
Total behavioural difficulties	7	beta	0.023	-0.10	0.15	0.709	5455
Emotional symptoms score	7	beta	0.006	-0.04	0.05	0.787	5462
Anxiety symptoms score	8	beta	-0.006	-0.05	0.04	0.809	5358
Depression	10	beta	0.004	-0.08	0.09	0.932	5434
IQ	8	beta	-0.004	-0.43	0.42	0.984	5290
Sleep initiation	7	OR	1.000	0.94	1.06	0.993	5479

Adolescents							
Frequency smokes cannabis	16	beta	-0.072	-0.15	0.01	0.078	1035
Ever smoked	14	OR	1.062	0.98	1.15	0.125	4145
Lifetime cigarettes smoked	18	beta	0.071	-0.02	0.17	0.142	1144
Conscientiousness	13	beta	-0.128	-0.3	0.05	0.151	4162
Conduct disorder	16	beta	0.031	-0.12	0.07	0.16	2871
Ever smoked cannabis	16	OR	0.949	0.88	1.03	0.182	3573
Lifetime cigarettes smoked	14	OR	1.099	0.95	1.27	0.193	1058
Emotional stability	13	beta	-0.109	-0.3	0.08	0.26	4224
Psychosis negative symptoms	16	beta	-0.096	-0.27	0.08	0.276	3513
Number of cigarettes smoked daily	23	beta	-0.003	-0.01	0	0.315	7841
Education	18	OR	0.955	0.87	1.05	0.324	2182
Number of cigarettes smoked weekly	23	beta	0.003	0	0.01	0.358	7841
Daily caffeine intake	13	beta	0.573	-0.65	1.8	0.359	3405
Eating disorder	13	OR	0.8	0.47	1.35	0.375	4256
Oppositional defiant disorder	15	beta	-0.011	-0.04	0.02	0.42	2948
Age of first cigarette	18	beta	-0.051	-0.18	0.08	0.427	1131
Exercise frequency	14	beta	0.009	-0.02	0.03	0.455	4270
Extraversion	13	beta	0.069	-0.13	0.27	0.503	4354
Depression	18	beta	-0.061	-0.24	0.12	0.509	3212

Depression symptom score	18	beta	0.043	-0.09	0.17	0.521	3303
ADHD	16	beta	-0.013	-0.05	0.28	0.526	2896
Depression	14	beta	0.039	-0.09	0.17	0.554	4574
Phobia symptom score	18	beta	0.006	-0.02	0.03	0.584	3293
Eating disorder	16	OR	1.078	0.78	1.5	0.627	3545
Autism	11	OR	0.934	0.69	1.27	0.637	5381
Life events	16	beta	0.004	-0.01	0.02	0.642	3378
Anxiety	18	beta	-0.006	-0.03	0.02	0.645	3293
Suicide attempt	16	OR	0.97	0.83	1.14	0.684	3263
Sleep maintenance	15	beta	0.005	-0.02	0.03	0.685	3419
Total behavioural difficulties	17	beta	-0.028	-0.18	0.12	0.709	4055
Lifetime cigarettes smoked	23	beta	0.007	-0.03	0.04	0.712	7841
Intellect	13	beta	-0.026	-0.19	0.14	0.759	4263
IQ	15	beta	-0.058	-0.48	0.36	0.786	3721
Sleep duration	16	beta	0.004	-0.03	0.04	0.803	3727
Psychosis positive symptoms	12	beta	-0.002	-0.02	0.01	0.804	4974
Ever smoked	18	OR	0.991	0.91	1.08	0.831	2402
Agreeableness	13	beta	0.015	-0.13	0.16	0.836	4279
Age of first cigarette	14	beta	0.002	-0.04	0.04	0.919	1064
BMI	17	beta	-0.006	-0.13	0.12	0.93	3606

Ever smoked	23	OR	0.997	0.91	1.09	0.94	2792
Sleep initiation	15	beta	0.017	-0.47	0.5	0.945	3627
Education	18	OR	1.005	0.79	1.28	0.965	2360
Psychosis positive symptoms	18	beta	0	-0.02	0.02	0.968	3403
Psychosis positive symptoms	17	beta	0.001	-0.06	0.06	0.981	4073
PTSD	15	beta	0	-0.01	0.01	0.983	4009

Table 5.5: Associations between maternal alcohol PRS and offspring mental health phenotype (intergenerational)

Phenotype	Age	Type	OR/beta	lowerCI	upperCI	pvalue	*n*
Intellect	13	beta	-0.209	-0.38	-0.04	0.016	3956
Daily caffeine intake	8	beta	0.774	-0.04	1.59	0.064	4067
Exercise frequency	14	beta	0.022	0.00	0.05	0.086	3969
Conscientiousness	13	beta	-0.158	-0.34	0.02	0.090	3863
Depression	10	beta	0.073	-0.02	0.16	0.112	4885
Education	18	OR	0.927	0.84	1.02	0.122	2038
Depression	14	beta	0.100	-0.03	0.23	0.142	4250
Ever smoked	23	OR	1.065	0.98	1.16	0.145	2610
IQ	15	beta	0.320	-0.13	0.77	0.161	3450
BMI	17	beta	-0.087	-0.22	0.05	0.214	3353
Total behavioural difficulties	7	beta	0.079	-0.05	0.21	0.217	5135
BMI	7	beta	-0.035	-0.09	0.02	0.223	5032
Age of first cigarette	14	beta	-0.026	-0.07	0.02	0.246	970
Ever smoked	14	OR	1.044	0.96	1.13	0.271	3876
Frequency smokes cannabis	16	beta	-0.041	-0.12	0.03	0.285	960
Education	18	OR	1.132	0.89	1.44	0.286	2204
Extraversion	13	beta	0.109	-0.10	0.32	0.309	4046
Lifetime cigarettes smoked	14	OR	1.078	0.92	1.26	0.316	965

Sleep initiation	15	beta	-0.266	-0.79	0.25	0.316	3355
Agreeableness	13	beta	-0.076	-0.23	0.07	0.323	3983
Anxiety	18	beta	-0.013	-0.04	0.01	0.331	3051
Sleep maintenance	7	OR	0.971	0.91	1.04	0.348	5129
Conduct disorder	7	beta	0.009	-0.011	0.029	0.378	5014
Hyperactivity symptoms	7	beta	0.008	-0.01	0.03	0.385	4918
Psychosis positive symptoms	18	beta	-0.007	-0.02	0.01	0.414	3180
Conduct disorder	16	beta	-0.02	-0.03	0.06	0.437	3538
Ever smoked	18	OR	1.034	0.94	1.13	0.455	2239
Lifetime cigarettes smoked	18	beta	-0.036	-0.13	0.06	0.460	1041
Sleep maintenance	15	beta	0.010	-0.02	0.04	0.461	3179
Age of first cigarette	18	beta	-0.050	-0.18	0.08	0.465	1038
Lifetime cigarettes smoked	23	beta	0.013	-0.02	0.05	0.476	7727
Depression	18	beta	-0.068	-0.26	0.12	0.483	3015
Eating disorder	16	OR	1.098	0.81	1.49	0.518	3310
Psychosis positive symptoms	17	beta	0.018	-0.04	0.08	0.542	3765
Autism	11	OR	1.082	0.79	1.48	0.593	4910
Ever smoked cannabis	16	OR	1.021	0.94	1.11	0.617	3335
Psychosis negative symptoms	16	beta	0.045	-0.14	0.23	0.626	3273
Sleep initiation	7	OR	1.014	0.95	1.08	0.645	5152

Total behavioural difficulties	17	beta	0.033	-0.13	0.19	0.684	3748
Number of cigarettes smoked weekly	23	beta	0.001	-0.01	0.01	0.726	7727
Eating disorder	13	OR	1.080	0.67	1.73	0.731	3956
Sleep duration	7	beta	-0.004	-0.03	0.02	0.748	5129
Phobia symptom score	18	beta	0.004	-0.02	0.03	0.750	3051
Life events	16	beta	-0.003	-0.02	0.02	0.762	3148
Specific phobia	10	OR	1.036	0.80	1.34	0.766	5102
IQ	8	beta	0.069	-0.40	0.53	0.771	4675
Handedness	10	OR	1.012	0.92	1.11	0.778	4849
Number of cigarettes smoked daily	23	beta	-0.001	-0.01	0.00	0.789	7727
Psychosis positive symptoms	12	beta	-0.002	-0.02	0.01	0.809	4568
Oppositional defiant disorder	7	beta	-0.003	-0.02	0.02	0.813	4492
Sleep duration	16	beta	-0.004	-0.04	0.03	0.817	3456
Emotional stability	13	beta	0.022	-0.18	0.23	0.832	3901
Anxiety symptoms score	8	beta	0.004	-0.04	0.05	0.860	4995
Daily caffeine intake	13	beta	-0.099	-1.28	1.08	0.870	3157
Suicide attempt	16	OR	1.012	0.86	1.19	0.875	3076
Depression symptom score	18	beta	0.009	-0.13	0.15	0.894	3059
Emotional symptoms score	7	beta	0.003	-0.04	0.05	0.904	5141
ADHD	16	beta	-0.002	-0.04	0.04	0.908	3566

PTSD	15	beta	0.000	-0.01	0.01	0.957	3722
Life events	7	beta	0.000	-0.02	0.02	0.989	5169
Oppositional defiant disorder	15	beta	0.000	-0.03	0.03	0.999	2759

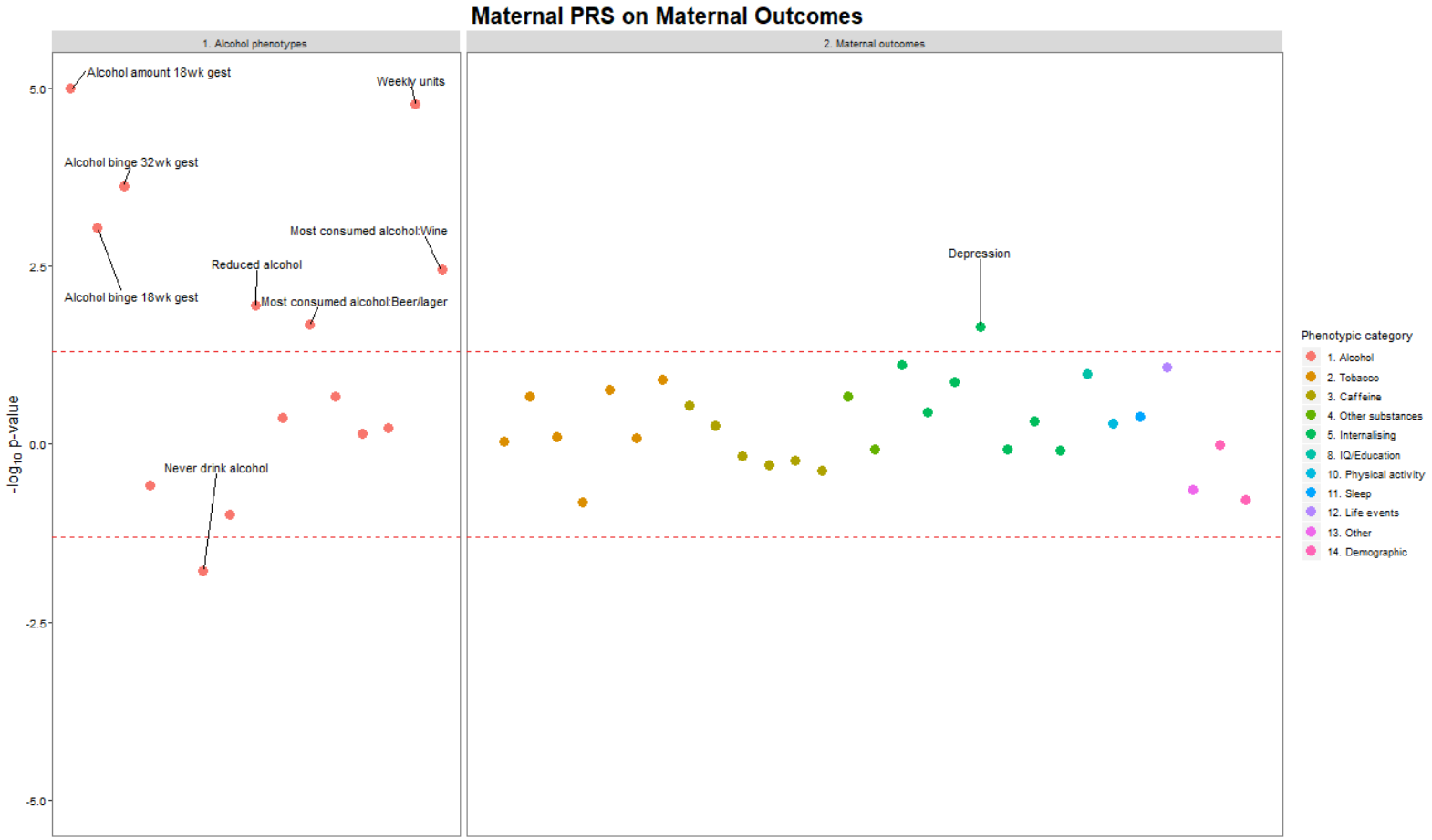

Figure 5.5: Maternal PRS for alcohol and maternal alcohol exposures and mental health phenotypes. Each datapoint represents an individual phenotype, colour coded by phenotypic category. Main effects are indicated by labelled phenotypes

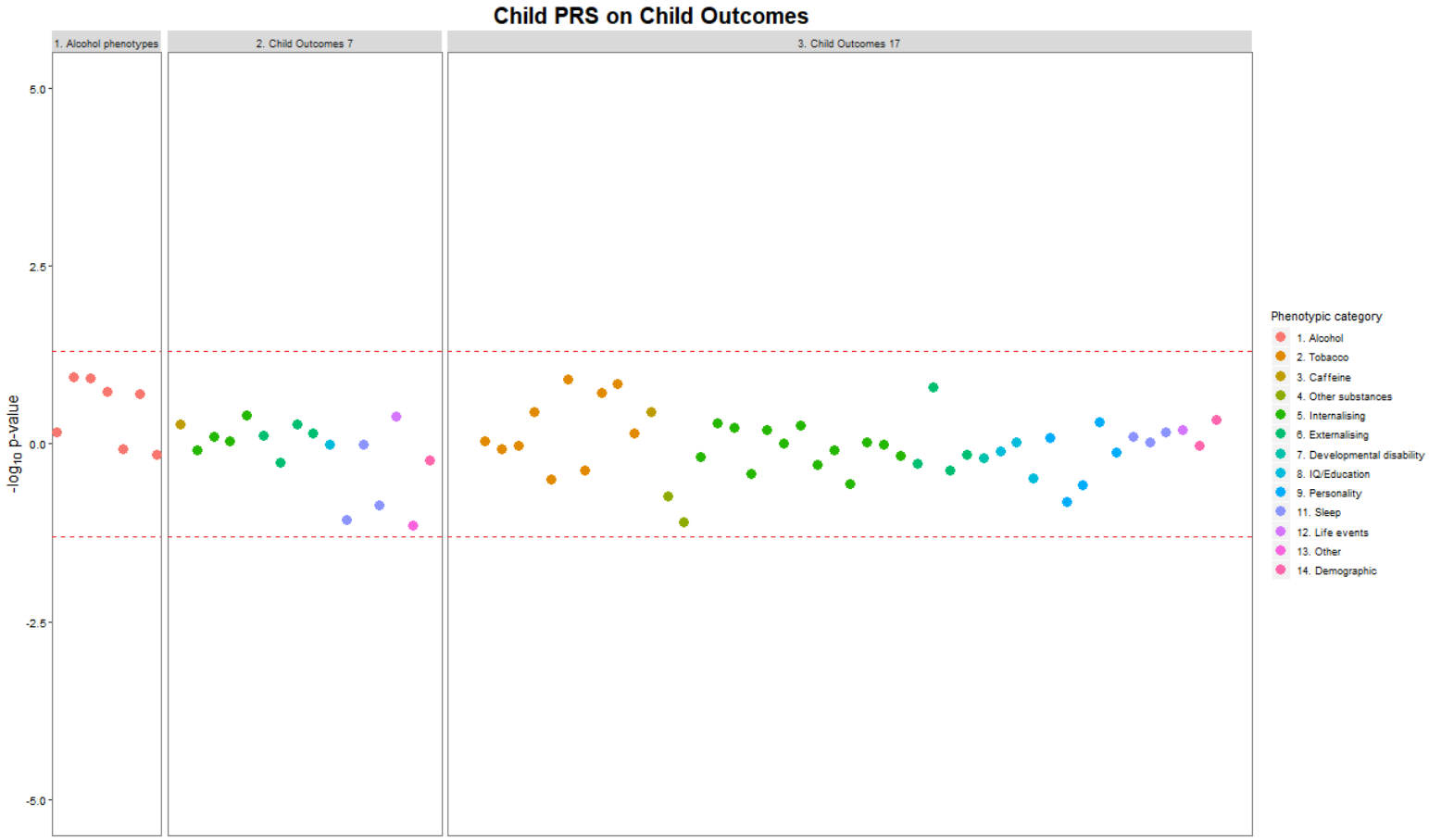

Figure 5.6: Child PRS for alcohol and child alcohol exposures, mental health phenotypes at pre-drinking age and drinking initiation age. Each datapoint represents an individual phenotype, colour coded by phenotypic category. Main effects are indicated by labelled phenotypes

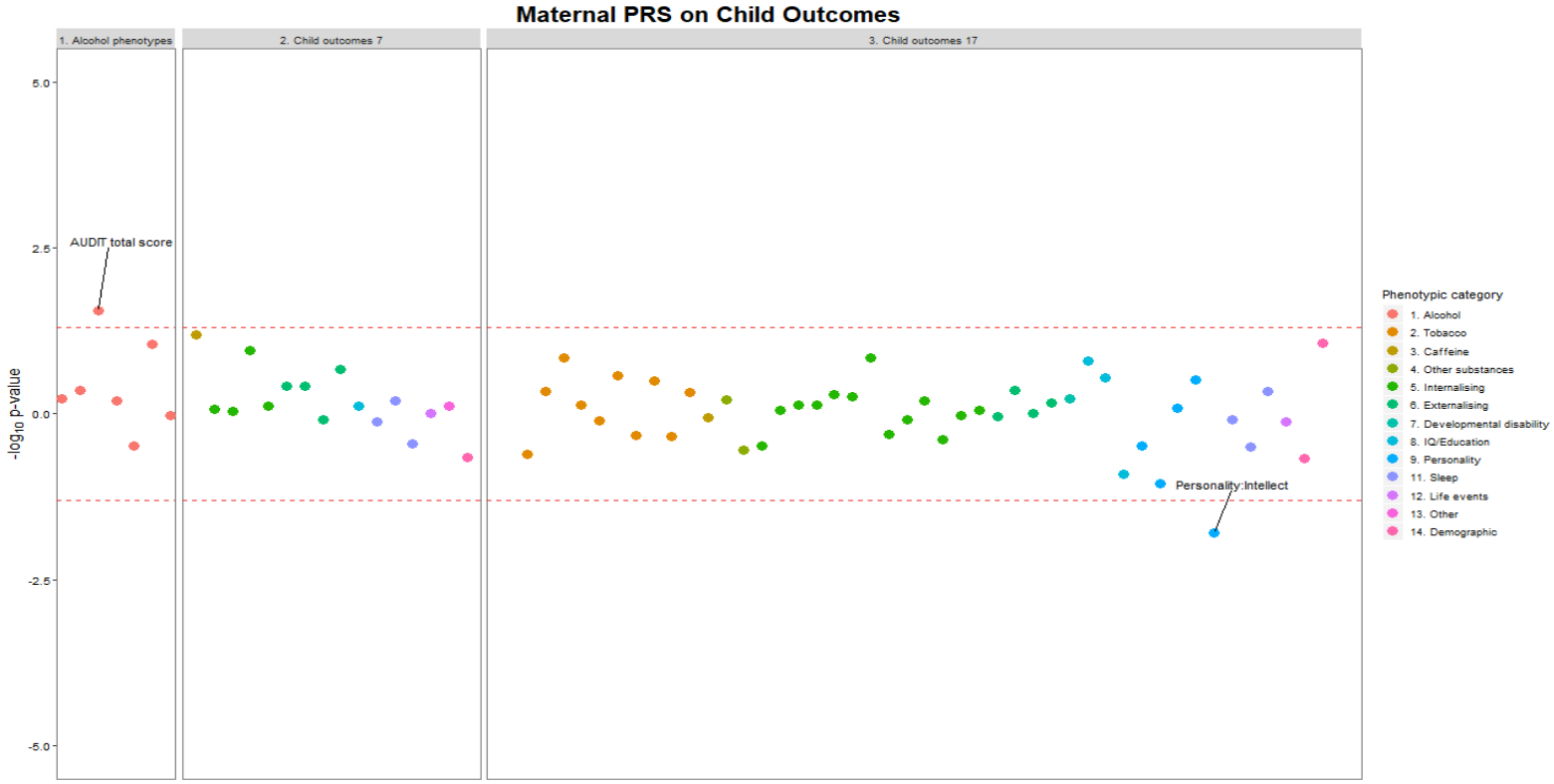

Figure 5.7: Maternal PRS for alcohol and child alcohol exposures, mental health phenotypes at pre-drinking age and drinking initiation age (intergenerational). Each datapoint represents an individual phenotype, colour coded by phenotypic category. Main effects are indicated by labelled phenotypes

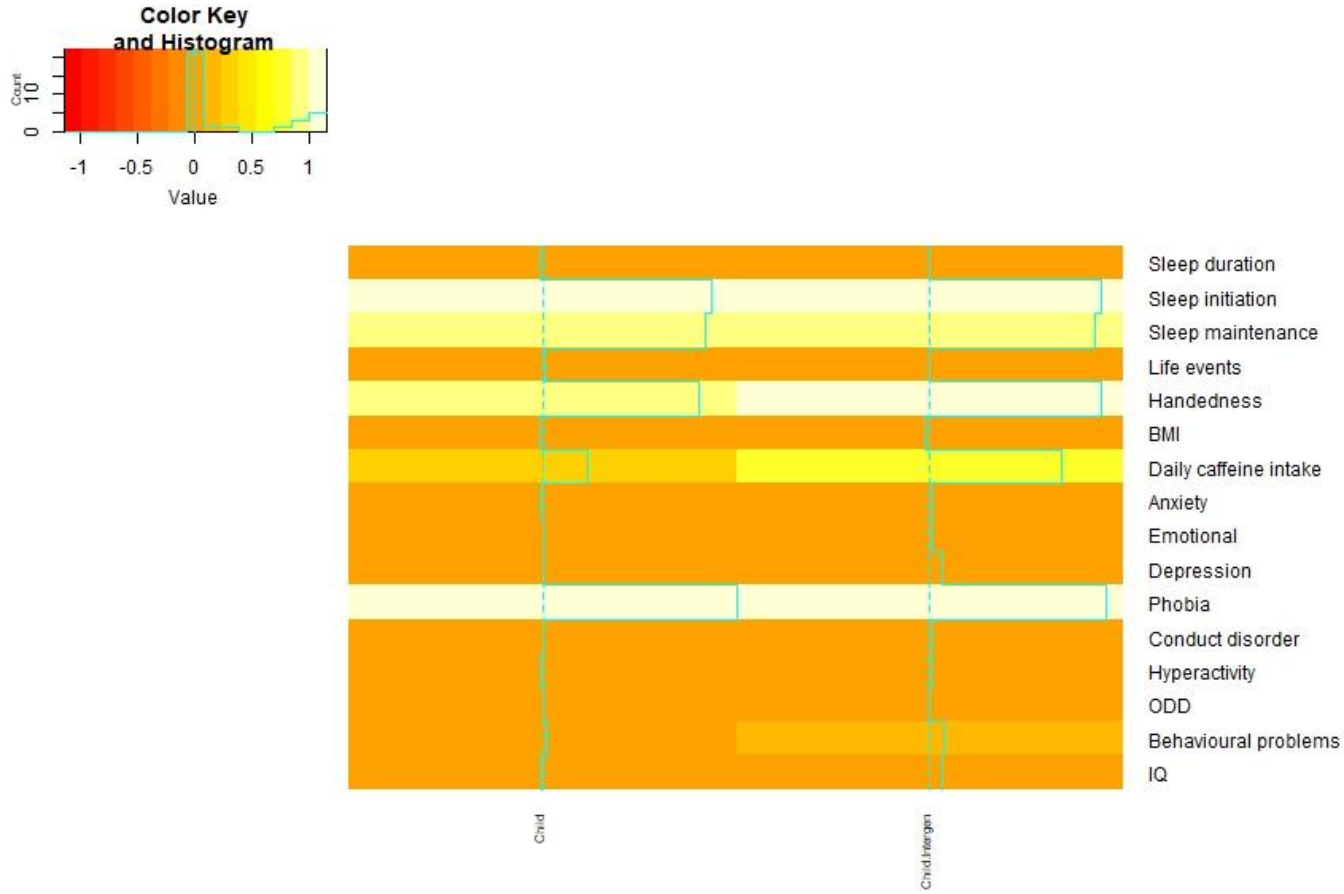

Figure 5.8: Heatmap showing strength of associations for child and intergenerational analyses, at pre-drinking age (~age 7)

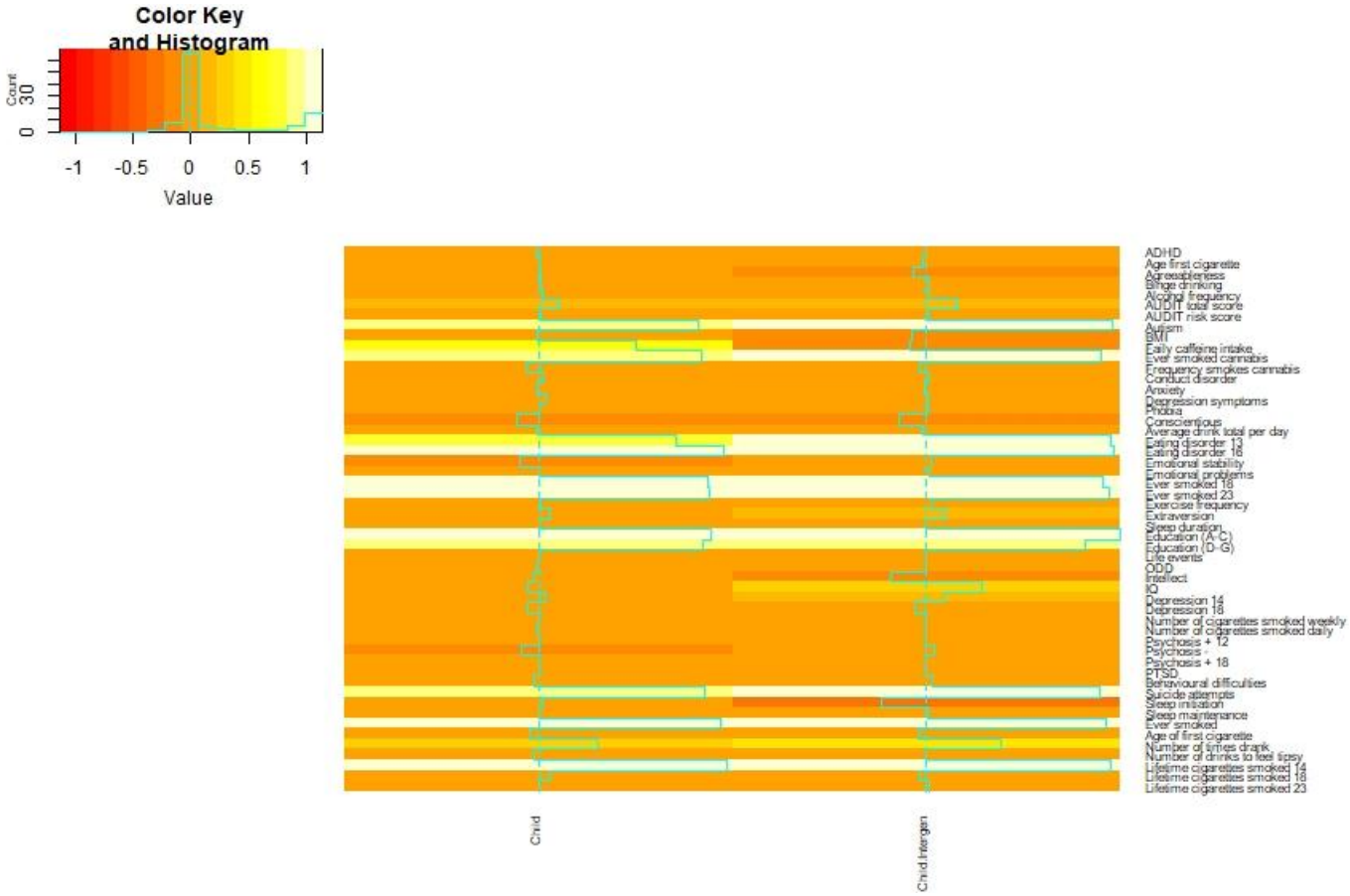

Figure 5.9: Heatmap showing strength of associations for child and intergenerational analyses, at drinking initiation age (~age 17)

5.4 Discussion

In this chapter I used a PheWAS design to investigate the effects of both maternal and offspring PRS for increased alcohol consumption on multiple mental health outcomes, in three separate pathways. These analyses were, maternal PRS on maternal phenotypes in pregnancy, child PRS on child phenotypes and maternal PRS on offspring phenotypes. The PRS for alcohol use were first validated to check if they were related to alcohol use phenotypes in ALSPAC for both mothers and offspring. I found the maternal PRS for increased alcohol use were associated with increased maternal alcohol use in pregnancy phenotypes, and therefore validated as being reliable instruments for alcohol consumption within this sample of pregnant women. Subsequent analyses tested which if any of the included maternal mental health phenotypes during pregnancy were associated with maternal PRS for alcohol use, and found an association with increased maternal depression at 32 weeks gestation. This may mean that the genetic variants for increased alcohol use has pleotropic effects and influences the outcome through pathways other than through alcohol use. Alcohol abuse is shown to have high comorbidity with mental health problems (L. Burns & Teesson, 2002; Kessler et al., 1997), and therefore genetic variants which are known to influence alcohol consumption may also influence negative mental health outcomes. Further investigation is required to disentangle what pathways of effect are being shown within this chapter.

Within each of these analyses I sought to test the estimations and did not expect to find a large magnitude of effect, particularly when investigating offspring PRS for alcohol consumption and offspring phenotypes. This is because offspring would have 50% shared genetic data with their mother, and I would therefore expect the effects found to be roughly halved compared to the mother's analyses. However, when validating offspring PRS for alcohol consumption against their alcohol use phenotype, I found there were no associations, suggesting that the child's own PRS for increased alcohol use are not a reliable instrument within this sample. However, this was not unexpected. The PRS created within the current study were based on multiple cohorts used within a previous large GWAS (Liu et al., 2019) such as 23andMe, and UK Biobank whose average age of participants is much older than that of the ALSPAC adolescents used in this study. The older age of the participants the original scores were based on would mean that their drinking patterns are fully established and relatively stable in comparison to younger cohorts (Maggs & Schulenberg, 2004). Previous studies have shown the prevalence of alcohol use to increase over time from adolescence to adulthood (Paavola, Vartiainen, & Haukkala, 2004). It is likely that if the ALSPAC cohort continued into late adulthood without attrition and this analysis was repeated, I would expect to see the PRS validated

for the offspring, comparable to the validation shown from the current study for the mothers PRS. The offspring PRS for alcohol use was also not shown to be associated with any of the mental health outcomes in the offspring subpopulation. Two separate timepoints of mental health and alcohol measures were included within the offspring subpopulation, ages ~18 and ~7. An earlier age group was included to be able to disentangle the influence of genetic variants from alcohol use initiation. If any effects were shown for the younger age group when the offspring were unlikely to have started consuming alcohol yet, I could be fairly certain that these effects were in fact due to the PRS and not through environmental influences of own alcohol use. If effects were shown for the negative control analyses at an earlier age of outcome, I would be showing pleiotropic effects. In the absence of offspring PRS predicting their alcohol use, I was therefore unable to explore this in depth. Offspring PRS for increased alcohol use was not associated with offspring mental health phenotypes independent of maternal drinking. However, as I did not find offspring PRS for alcohol use to be associated with offspring alcohol phenotypes I am fairly certain that I am not seeing pleiotropic effects. There is however still a chance that there could be a non-transmitted pleiotropic effect from maternal PRS for alcohol use to offspring phenotypes. Future work could utilise trios (where mother, father and offspring genotype are included) to focus on the influence on non-transmitted haplotypes. If an effect was shown within this design, it would suggest that it is not due to transmission of shared genotype from mother to child. But may instead be through environmental effects, as well as effects from the fathers genotype. The sample sizes for offspring phenotypes were also relatively small as they were mostly included at aged 18 and above (*n* = ~4000*)*, dropping from more than 15000 participants at the start of the cohort. Larger sample sizes would increase the statistical power of these analyses and potentially identify smaller effects.

In the final subgroup, I investigated the effect of maternal PRS for increased alcohol use on offspring mental health phenotypes in intergenerational analyses. This subpopulation would help to elucidate potential mechanisms behind any effects shown. Within the intergenerational analyses, maternal PRS for increased alcohol use were associated with offspring increased alcohol risk at age 18 as measured by the AUDIT measure, but no other alcohol measures. Similarly to findings from the offspring's own PRS subpopulation, this again may be due to the younger age of the adolescents within this subpopulation not yet having fully established drinking behaviours, and potentially again due to the smaller sample size available for the alcohol phenotypes. Maternal PRS for increased alcohol use also had an effect of decreased scores for intellectual ability. As I had already shown maternal PRS to be validated as a measure of increased maternal alcohol use, this would suggest that offspring of mothers with increased levels of PRS for

alcohol use would have likely been exposed to greater amounts of PAE. Although this cannot be explored statistically with the present data, self-reports of alcohol use can often be underreported (Feunekes, van 't Veer, van Staveren, & Kok, 1999). It has already been suggested that mothers often underreport prenatal alcohol use (Wurst et al., 2008), potentially due to fear of stigma. Many mothers are also not aware that they may be pregnant until later into their term, and if they are already consuming higher levels of alcohol use it is likely that any pregnancy may have had high levels of PAE on the developing fetus.

In subsequent sensitivity analyses to adjust for multiple comparisons, Bonferroni tests were used, and any effects previously seen were attenuated. However, Bonferroni correction is a very conservative test, and it was not unexpected that it may lead to a high rate of false negatives. Permutation testing showed the findings within the maternal subpopulation were the only effects that remained. The intergenerational effects previously seen in the main analyses did not persist. This could have been because the effects previously shown in the main analyses were already small as PRS are known to explain a small amount of phenotypic variance.

The strengths of these analyses are shown within their multiple subpopulation design, utilising both maternal and offspring PRS within each. This design allows more certainty of what may be causing any observed effects, either own alcohol consumption, or maternal alcohol exposure. Through using a PheWAS design, I included all available mental health phenotypes from ALSPAC that fit the criteria defined within the methods. The inclusion of 90 mental health phenotypes allowed a more comprehensive investigation of possible influences of alcohol use and may have shown pathways to different subtypes of mental health that we were less certain we would see an effect for. The SNPs which my PRS for increased alcohol consumption were derived from were based on a large sample size of over 1 million individuals. This large sample size is likely to have created a genetic score with increased statistical power to identify alcohol consumption compared to previous GWASs of alcohol use (Clarke et al., 2017).

There may, however, be limitations also. Although a PheWAS is an advancement in establishing potential associations and pleiotropy from genetic variants, the benefits of a PheWAS are reliant on how well the phenome can be defined. Due to the suitability of the phenotypes within the cohort I was investigating, I conducted a targeted PheWAS. This meant that I may have also missed out on other phenotypic associations which could have been present in ALSPAC but were not included as outcome phenotypes. However, as I limited my search strategy to include only mental health phenotypes, I am fairly certain that the current study is representative of the possible mental health phenotypic

associations with PRS for increased alcohol use that are available within ALSPAC. Another potential limitation is due to the original cohorts that the SNPs for increased alcohol consumption were derived from (Liu et al., 2019). This paper included multiple cohorts within their analyses, including ALSPAC. This means that there is an overlap between participants included in deriving the SNPs from the Lui and colleagues' paper, and the analyses in the current study. However, the original paper included a sample size of 8913 participants from ALSPAC out of a total of 1.2 million included participants overall. This means there is a slight, albeit very small, overlap between participants included with the same confounding structures in the GWAS effects and the current analyses.

When trying to ascertain exactly what the underlying mechanisms may be from these findings, it is difficult to be certain. The intergenerational analyses would suggest a causal pathway from mothers' alcohol PRS to offspring outcomes. However, it could be due to any of the following. It is likely that there is an intergenerational effect of maternal alcohol use on offspring outcomes. Increased maternal alcohol use postnatally may also be having an influence on offspring outcomes. This may be due to differences in how the mother interacts with her child or differences in parenting styles (Lieb et al., 2000). As discussed, there may be intergenerational pleiotropic effects of maternal PRS acting on their child's mental health outcomes. The findings could also be due to another form of pleiotropy of a shared genetic confounding between increased alcohol use and mental health. However, as previously discussed as I did not observe any effects between child's own PRS for alcohol use and child mental health outcomes this is unlikely.

The results I have reported could suggest intergenerational effects, or it could be that the study was underpowered to detect a true effect. A PheWAS is an exploratory method which allows investigation of potential pathways to harm. However, as discussed it cannot truly disentangle if any effects shown are evidencing causal pathways or are due to pleiotropy. A PheWAS does not empirically test the assumptions that would be required to investigate this further. Therefore, further testing using Mendelian Randomization could help to formally test the findings from this chapter.

5.5 Chapter summary

In this chapter I conducted a PheWAS to investigate the effects of (both maternal and offspring) PRS for increased alcohol use on multiple mental health phenotypes, in three separate subpopulations. I showed that the maternal PRS for alcohol use were validated within mothers during pregnancy and were also shown to influence maternal

prenatal depression. In intergenerational analyses, maternal PRS for alcohol use were also shown to be associated with offspring increased hazardous drinking levels. Maternal PRS were also associated with decreased scores of offspring intellectual ability. We may be observing intrauterine effects of maternal alcohol use on offspring, however, replication using larger sample sizes is required.

Each chapter so far has focused on cross-sectional analyses and comparisons of mental health between varying ages. The following chapter will utilise statistical analyses which allow measurement of change over time and population average trajectories of adolescent mental health.

Chapter 6 Association of maternal alcohol use in pregnancy with offspring depressive symptoms and conduct problems

The results described in previous chapters suggest that the amount of alcohol consumed by mothers during pregnancy is associated with negative offspring mental health outcomes. Much of the previous research within this area has focused on offspring ages of early childhood, this thesis aimed to investigate older ages of offspring to ascertain if any associations shown in early childhood may persist into late adolescence and early adulthood. However, each chapter has so far measured outcomes from individual timepoints. Statistical models exist which estimate the trajectory of variables over multiple timepoints (e.g. increasing age), as well as establishing classes (categories) of trajectories that may exist within a phenotype. The following chapter again explores mental health phenotypes but this time using longitudinal data from repeated measures, by using both longitudinal modelling and latent class analyses to investigate the effects of maternal PAE on offspring depression and conduct problems across multiple timepoints. If critical timepoints are found within these analyses, it could provide insight into sensitive timepoints in adolescence where interventions could be targeted at to reduce mental health problems.

6.1 Introduction

The previous chapters have shown maternal PAE to be associated with offspring mental health problems such as depression, even within older offspring age groups. This is comparable to previous research which has also shown associations within younger offspring age groups (Kendler et al., 2013; O'Connor & C Kasari, 2000; Ware et al., 2013), as previously discussed. Chapter Two highlighted the disparities between strength and direction of associations between studies, which are likely due to differences in study methodology, such as cohort demographics and alcohol and mental health measures used. However, there is also evidence to suggest that outcomes may vary at different ages (Moffitt, Caspi, Dickson, Silva, & Stanton, 1996). By using longitudinal data, we can increase precision around a static measure and measure the way in which an outcome can change. If a critical age is shown for the development and change of a mental health outcome over adolescence, this information can then be used to tailor potential interventions to reduce harm at specific timepoints. By only measuring offspring mental

health outcomes at a single timepoint, we may be not be measuring any changes that could occur over time, or not be evident at certain ages.

6.1.1 Trajectory analyses

It is common to study associations for different age groups at a single timepoint and compare the strength of evidence between different ages for comparison of prevalence. However, this does not capture how a behaviour may change over time. Trajectory analyses have been used within multiple research areas to investigate how health behaviours may present at different timepoints across the life course, peak and change over time within different populations. Understanding potential trends in health behaviours, can therefore aid in the design and implementation of interventions, by highlighting vulnerable time periods of risk. Longitudinal cohort studies are an excellent resource for conducting trajectory analyses as they often contain rich data from repeated measures at increasing ages. The analysis of these models is useful in understanding the complexity of health behaviours and what may be influencing their manifestations. For example, trajectory analyses have been used previously to study how substance use and abuse may vary from adolescence to adulthood (Britton et al., 2015; Maggs & Schulenberg, 2004). By investigating the age and frequency at which adolescents begin consuming alcohol, it is possible to identify the potential life stages of alcohol initiation and use. The identification of developmental trends mean we are then able to predict the average trend for future alcohol use, as predicted by past alcohol patterns. Trajectories have also previously been investigated within varying populations for mental health problems. Depressive symptoms have been shown to increase from childhood through to adolescence (Natsuaki, Biehl, & Ge, 2009; Rice et al., 2018). Such findings highlight the potentially time sensitive periods of development of depressive symptoms. However, previous research has not investigated how these depression trajectories may change over time in relation to PAE.

6.1.2 Latent class analysis

Latent class analysis (LCA) is another analytical method used to investigate patterns in behaviours and again relies on longitudinal or repeated measurements. Conventional growth modelling assumes that all included individuals come from a singular population and presents a single trajectory over time which should represent its population (Jung & Wickrama, 2008). However, it is recognised that there are instances where individual phenotypes may consist of very distinct categories which need to be accounted for separately to estimate appropriate changes over time. LCA recognises that

there may be different categories of an observed phenotype within specific subpopulations (Assanangkornchai, Li, McNeil, & Saingam, 2018; M. Taylor et al., 2017) and separates these classes to show their individual varying trajectories across time. LCA uses repeated measurements within a dataset to find groups or categories within a phenotype, by establishing patterns of associations within a behaviour. These can then be investigated as a potential exposure or outcome (Agrawal, Lynskey, Madden, Bucholz, & Heath, 2007; Heron et al., 2012). By only measuring behaviours from a single timepoint we may be prone to missing developmental trajectories, and how these trajectories may differ for distinct classes within each behaviour (Moffitt, 1990). For example, Barker and Maughan (Barker & Maughan, 2009) identified heterogeneity in the development of conduct problems in childhood and measured four distinct classes of conduct problems in children within the ALSPAC cohort. The four conduct problem classes shown by Barker and Maughan within ALSPAC have been replicated and validated (Heron et al., 2013; Kretschmer et al., 2014). These statistical methods can be taken a step further to not only highlight patterns of change and growth in health behaviours within subpopulations, they can be used to assess the influence of different predictors on distinct classes of behaviours (Liao et al., 2019; MacKinnon, Kingsbury, Mahedy, Evans, & Colman, 2018).

Latent class growth analysis (LCGA) is a form of Growth Mixture Modelling, in which the variance included within each derived category are fixed to zero. The methods behind this form of modelling have been developed extensively by Nagin and colleagues (Nagin & Land, 1993). LCGA will be used within this chapter to model the conduct disorder classes, as this allows each class to be defined before the addition of potential covariates. A debate for using LCGA is given in how to establish the most suitable number of classes for an outcome. However, reliable methods are used to validate the most appropriate number of classes. For example, the Bayesian information criteria (BIC) is a method for testing the efficiency of each model, with the model showing the smallest BIC most accurately predicting the number of classes within the data (Jung & Wickrama, 2008). Latent classes have been advantageous in measuring many other distinct outcomes such as alcohol problems as well as cannabis use within ALSPAC (Heron et al., 2012; M. Taylor et al., 2017).

Depression often manifests in adolescents, but how this may then vary over time could be influenced by prenatal alcohol use. Previous research has investigated the effect of parental alcohol use on later measures of offspring depression and behavioural problems such as conduct problems (Mahedy et al., 2017). Mahedy and colleagues focused on postnatal alcohol use when offspring were 5 years of age and found no evidence for an association between parental (maternal and partner) alcohol use and

offspring depression or conduct problems in the ALSPAC cohort. This is unsurprising when compared to my findings from Chapter Four, where I found parental postnatal alcohol use to not be related to offspring mental health problems after controlling for possible confounding influences. Chapter Three showed associations between maternal PAE and offspring mental health which persisted after adjustment for potential confounders. These variations in findings suggest PAE may have more of an effect on offspring mental health than postnatal alcohol use, however, previous research has yet to assess the influence of PAE on trajectories and latent classes of offspring mental health. This chapter replicated methods used by Mahedy and colleagues by investigating the influence of parental alcohol use on offspring trajectories of depressive symptoms and latent classes of conduct problems. However, instead of postnatal alcohol use, I use PAE as the exposure of interest.

Each analytical chapter so far has been conducted using outcome data from individual timepoints. An advantage of the ALSPAC cohort used within this thesis is that it has measures recorded at multiple timepoints across the life course. Repeated measurements of the same outcome across increasing ages allow the investigation of phenotypic population-averaged trajectories within a dataset, and how included covariates may affect their trajectories in relation to earlier measured exposures, such as maternal PAE. Repeated measures across time can provide more robust measures in comparison to individual timepoints. Repeated measures do exist for maternal alcohol exposures also, however, the analyses conducted within this chapter was designed to address how mental health outcomes may change over time in relation to PAE. Therefore, this chapter aims to measure the latent classes for conduct problems within childhood, as well as the developmental trajectories of depressive symptoms within the ALSPAC cohort. To subsequently investigate if maternal PAE is associated with changes in depressive symptoms, and with distinct classes of conduct problems throughout childhood and adolescence. If critical timepoints are found within these analyses, it could provide insight into sensitive periods in adolescence where interventions could be targeted at to reduce mental health problems for offspring at risk.

6.2 Methods

6.2.1 Study population

The ALSPAC cohort as described in Chapter Three was again used for the current chapter. Pregnancies were excluded if they were triplets or quadruplets, and siblings were also removed from the analyses.

13,195 mothers completed the self-report questionnaires on PAE at 18 weeks gestation. As each of the confounders were measured during pregnancy there is minimum missing data. Counts for PAE and included confounders have been detailed in previous chapters.

6.2.2 Measures

Exposures. Maternal alcohol consumption during pregnancy was measured by frequency of drinking alcohol using the same measure as described in Chapter Three. At 18 weeks gestation mothers were asked the frequency and amount of alcohol they had consumed within the past 3 months. Response categories were never, <1 glass per week, 1+ glass per week, 1-2 glasses a day, 3-9 glasses a day and ≥10 glasses a day. Due to sample size restrictions in terms of available complete case data across repeated measures, the response categories were condensed to never, <1 glass per week and 1+ glass per week.

Outcomes. Adolescent depressive symptoms were measured using the SMFQ. This is a self-report measure questionnaire that participants completed via a postal questionnaire or during ALSPAC clinic attendance, at four separate timepoints about the occurrence of their depressive symptoms over the past two weeks (aged: 12 years 10 months, 13 years 10 months, 16 years 6 months, 17 years 10 months). The SMFQ is comprised of 13 items, with summed scores that can range between 0-26. Higher scores on this questionnaire indicate higher amounts of depressive symptoms. Scores >11 have been previously used as a cut off for depression (Turner, Joinson, Peters, Wiles, & Lewis, 2014). Validity and reliability of the SMFQ has been calculated in previous studies, indicating strong internal consistency of Cronbach's alphas >85 (Thabrew, Stasiak, Bavin, Frampton, & Merry, 2018). Validity of the SMFQ also showed strong correlations with the Mood and Feelings Questionnaire (MFQ) and the clinician-rated Children's Depression Scale-Revised, with all 13 items indicating item total correlations of at least .50 across all three measured timepoints. Depressive symptoms have been shown to differ across gender during adolescence, I therefore utilised the *knownclass* option within Mplus to estimate separate latent growth models for males and females within the data. This method constrains the association between the exposure with the intercept and slope to be equal, and the variances to be equal to then provide a single estimate, as well as maximising power. The residual variances are therefore constrained within time, yet freely estimated across gender (Mahedy et al., 2017).

Conduct problems were measured using the conduct problems subscale within the SDQ at six separate timepoints (4, 7, 8, 10, 12, and 13.5 years), through maternal

report. The validity and reliability of this measure have been previously reported (see Appendices 6.2-6.3). The conduct problem subscale consists of five items: 1) ”Often has temper tantrums or hot tempers”; 2) “generally obedient, usually does what adult request”; 3) “Often fights with other children or bullies them”; 4) “Often lies or cheats”; 5) “Steals from home, school or elsewhere”. Based on the national established norms for children in England and Wales (Meltzer, Gatward, Goodman, & Ford, 2003), binary cut offs were used with a threshold of 4 or more indicating high risk of conduct problems. Conduct disorder has high heterogeneity in the presentation of symptoms across childhood and adolescence. Barker and Maughan (Barker & Maughan, 2009) derived four separate classes of child conduct disorder within ALSPAC which reflect the heterogeneity of conduct problems; early onset persistent, childhood limited, adolescent onset and low conduct problems. These classes have been used and validated in subsequent studies (Gage et al., 2014; Mahedy et al., 2017) to investigate the developmental nature of conduct disorder across time, and have shown a four class model to be the most representative of the available classes for this sample over greater or fewer classes (Barker & Maughan, 2009; Mahedy et al., 2017). For this chapter I estimated the same four latent classes of conduct problems using a Growth Mixture Model (GMM). Barker and Maughan (Barker & Maughan, 2009) have previously shown gender-invariant models did not give adequate fit to the data, I have therefore investigated trajectories of conduct problems for the whole sample and did not split the analyses by gender. Subsequent analyses were conducted on anyone who had complete data on conduct problems for at least one timepoint (n = 7218).

Confounders. Potential confounding factors associated with maternal PAE and offspring mental health were included in the analysis, again kept congruent from those used in previous chapters to be able to compare models. Mother’s socioeconomic position (professional/managerial or other) measured during pregnancy, income (divided into quintiles) measured at age 3 and 4 years, home ownership (mortgage/non-mortgage) measured at 8 weeks gestation, marital status (married or not) measured at 8 weeks gestation, maternal education (university degree/<university degree), sex, parity (first born, 2+ born), maternal tobacco (yes/no) and illicit drug use (yes/no) in months 1-3 of pregnancy, and maternal depression at 18 weeks gestation (scores >12 highly associated with a diagnosis of depression) measured by the Edinburgh Postnatal Depression Scale (EPDS) (Cox et al., 1996). These confounders were included to examine their effect of the association between maternal PAE and offspring outcomes, and were not included as main risk factors.

6.2.3 Statistical analyses

Adolescent depressive symptoms. Trajectories of adolescent depressive symptoms as measured by the SMFQ are already established within ALSPAC (Edwards et al., 2014; Rice et al., 2018) and are therefore are reported here only within Graph 6.1. After measurement of the estimated trajectories for depression across all timepoints, latent growth models were used of maternal PAE as a predictor of offspring depressive symptom trajectories using their intercept and slope factors at each timepoint using M*plus* Version 8. Depressive symptoms were included across all four timepoints. Analyses were conducted on individuals who provided information on at least two timepoints. The slope and intercept were calculated to indicate the linear relationship between age and depressive symptoms. The slope represents the gradient of the line and the intercept represents the location the line intersects the y axis. When investigating depressive symptoms, the latent variables (intercept and slope) are continuous variables. Therefore, when analysing the associations between PAE and offspring depressive symptoms, linear regressions are conducted. For model identification, intercept factor loadings were fixed at one and the slope factor loadings were fixed with a baseline of zero to account for time between assessments (months). As developmental trajectories of depression in adolescence have been shown to vary across sex (Edwards et al., 2014; Rice et al., 2018), separate latent growth models were generated for males and females using the *knownclass* command in M*plus*. This is in line with previous studies that have estimated a single covariate estimate by constraining the association between the covariate with intercept and slope to be equal across males and females (Edwards et al., 2014; Mahedy et al., 2017). Variances were constrained to be equal, while residual variances are freely estimated but constrained within time. Estimating a single covariate effect helps to maximise power. The betas were standardised to allow comparison of the strength of effect across the outcome. The betas were calculated by subtracting the mean from the outcome variable and dividing it by its standard deviation. Each one unit change is equivalent to one standard deviation change in the outcome.

Conduct problems. A latent class GMM was used to establish developmental trajectories of conduct problems across 6 timepoints. These were: early onset persistent, childhood limited, adolescent onset and low conduct problems. For all analyses, low conduct problems were used as the reference category. The auxiliary *(r3step)* command was used to avoid introducing bias into the model when establishing the classes. This method helps to adjust for classification uncertainty within the conduct problems classes and controls for measurement error. The most likely class membership is therefore obtained, and covariates were subsequently included during analyses. This approach has

been shown to produce less biased results compared to the traditional three step modal class approach (Heron, Croudace, Barker, & Tilling, 2015). Within the three-step approach, the initial step uses LCGA to derive the conduct problem classes. In the second step, offspring are assigned to the class they most likely belong to. This approach enables the classification of the most appropriate class membership from the posterior probabilities and does not force individuals into one definitive class, as well as including class uncertainty. The model fit estimates for the four conduct problem classes originally derived by Barker and Maughan (Barker & Maughan, 2009) and replicated for the current chapter are shown within the entropy value and BIC in Appendices 6.1. The four group class of conduct problems used showed a BIC value of 43198.07 and an entropy value of 0.79.

In the final step, multinomial logistic regression was used to examine the association between maternal PAE and the most likely class of offspring conduct problems including all auxiliary variables. This final step uses Vermunt's correction for classification errors where the classification errors from the first latent class model is used to adjust for the bias within the regression analyses (Vermunt, 2010).

6.3 Missing data and sensitivity analysis

Using data from a longitudinal cohort will result in attrition. However, an advantage of using longitudinal data is that full information maximum likelihood (FIML) estimation allows the inclusion of incomplete data. I therefore used full information maximum likelihood (FIML) to account for missing data, as using complete case data and not accounting for missing data could result in biased estimates (Sterne et al., 2009). FIML provides unbiased parameter estimates, by using the available variables to estimate a likelihood function for each individual. In contrast to multiple imputation, FIML estimates all parameters using all available data from repeated measures (Enders, 2001).

Analyses were conducted using Stata Version 15 and M*plus* version 8.

6.4 Results

6.4.1 Trajectories for depressive symptoms

Sample sizes for adolescent depressive symptoms ranged between 3790-5658 individuals (Table 6.1). Maternal alcohol use at 18 weeks gestation was completed for 6050 mothers. Complete data was available for 7768 individuals with recorded SMFQ measures for at least two timepoints for depressive symptoms.

Table 6.1: Descriptive statistics for depressive symptoms (SMFQ)

Age (years)	*N*	Mean
12.10	5658	3.46
13.10	5440	4.03
16.6	4050	4.77
17.10	3790	5.02

Trajectories for depressive symptoms in the ALSPAC dataset were explored for both sexes and is shown in Figure 6.1, with females and males following different trajectories. Females showed a change in depressive symptoms of a strong positive slope, and males showing a moderate positive slope as shown in previous research (Edwards et al., 2014; Rice et al., 2018). As previously discussed these depression trajectories have already been derived within the ALSPAC cohort and they are provided here only for reference only (Edwards et al., 2014).

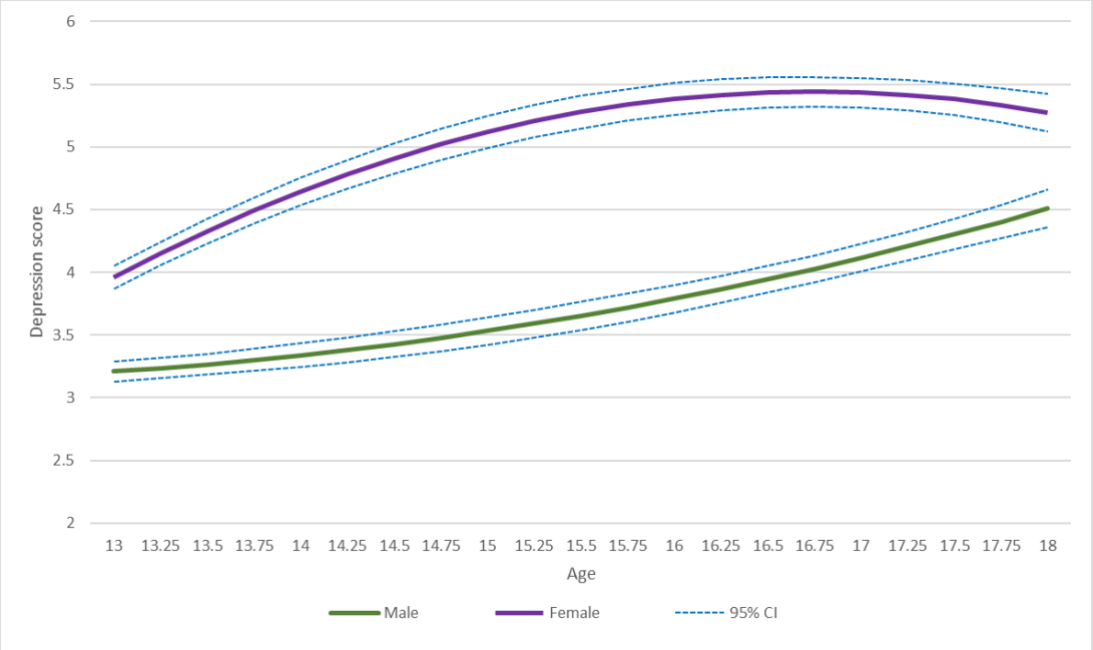

Figure 6.1: Average population trajectories for each gender of depressive symptoms, as measured in the SMFQ.

Latent growth modelling was used to derive the depression trajectories and linear regression models investigated associations between maternal PAE and adolescent depressive symptom trajectories. Linear regression analyses tested if associations between maternal PAE amounts and adolescent depressive symptoms differed between sexes. These analyses showed associations only between PAE and intercept for females in unadjusted models for depression for mothers consuming <1 glass per week ($\beta = 0.119$, 95% CI 0.03, 0.21) and 1 + glass per week ($\beta = 0.147$, 95% CI 0.03, 0.27), and not within males. These associations however did not remain after adjustment for potential confounding variables (Table 6.2). Further analyses tested the overall differences between males and females' depressive symptoms and PAE exposures and did not find evidence of any difference of association. This suggested that associations between PAE and offspring depression did not differ by sex (Table 6.2). As the associations did not differ by sex the analyses constraining associations on gender are presented below which are more parsimonious and have greater power (see Table 6.3). I found little evidence to suggest an association between amount of alcohol consumed by mothers at 18 weeks gestation and offspring slope and intercept of depressive symptoms in adolescence, in both unadjusted and adjusted models (see Table 6.3).

Table 6.2: Associations between maternal PAE and trajectories of offspring depressive symptoms by gender

	Intercept					Slope				
	Males		Females		Wald *p* value	Males		Females		Wald *p* value[b]
Unadjusted	β (95% CI)	*P*	β (95% CI)	*p*		β (95% CI)	*p*	β (95% CI)	*p*	
None	*Ref*		*Ref*			*Ref*		*Ref*		
<1 glass per week	0.001 (-0.09, 0.09)	0.988	0.119 (0.03, 0.21)	0.007	0.048	0.005 (-0.14, 0.15)	0.943	0.032 (-0.09, 0.15)	0.598	0.744
1+ glass per week	-0.003 (-0.12, 0.11)	0.959	0.147 (0.03, 0.27)	0.015	0.058	0.040 (-0.15, 0.23)	0.678	0.123 (-0.04, 0.28)	0.133	0.426
Adjusted										
None	*Ref*		*Ref*			*Ref*		*Ref*		
<1 glass per week	0.023 (-0.09, 0.14)	0.689	0.028 (-0.08, 0.13)	0.597	0.896	-0.017 (-0.18, 0.14)	0.597	0.030 (-0.11, 0.17)	0.680	0.664
1+ glass per week	0.100 (0.05, 0.26)	0.204	0.062 (-0.08,0.21)	0.401	0.837	0.023 (-0.19, 0.23)	0.401	0.136 (-0.06, 0.33)	0.166	0.400

Adjusted for: Socioeconomic position, income, home ownership, marital status, maternal education, parity, maternal tobacco use during 1-3 months of pregnancy, maternal depression 18 weeks gestation.

[b]Wald p value indicates the difference between gender within each category of maternal PAE

Table 6.3: Associations between maternal PAE and trajectories of offspring depressive symptoms

	Unadjusted (n=6050)				Adjusted (n = 4417)			
	Intercept *b* (95% CI)	*p*	Slope *b* (95% CI)	*p*	Intercept *b* (95% CI)	*p*	Slope *b* (95% CI)	*p*
<1 glass per week	0.063 (-0.01, 0.13)	0.074	0.023 (-0.08, 0.13)	0.662	0.027 (-0.06, 0.11)	0.533	0.008 (-0.10, 0.12)	0.882
1+ glass per week	0.071 (-0.02, 0.16)	0.117	0.094 (-0.04, 0.23)	0.179	0.086 (-0.03, 0.20)	0.148	0.087 (-0.06, 0.24)	0.255

Adjusted for: Socioeconomic position, income, home ownership, marital status, maternal education, parity, maternal tobacco use during 1-3 months of pregnancy, maternal depression 18 weeks gestation.

6.4.2 Conduct problems

The latent class analysis was based on 7218 children who had complete information for conduct disorder for at least one timepoint (Table 6.4). A GMM found four distinct classes of conduct problems to provide the best fitting model (see Figures 6.2-6.3). Within offspring who had conduct problems (CP), individuals had most likely class membership within one of four classes; childhood limited (11%), low (65%), adolescent onset (13%) and early onset persistent (11%). Figure 6.2 indicates the proportion of individuals that have conduct problems at each time point within each distinct conduct disorder class.

Table 6.4: Descriptive statistics for most likely conduct problem class membership

Conduct problem class	*n*	%
Low	912	13
Childhood limited	771	11
Adolescent onset	812	11
Early onset persistent	4723	65

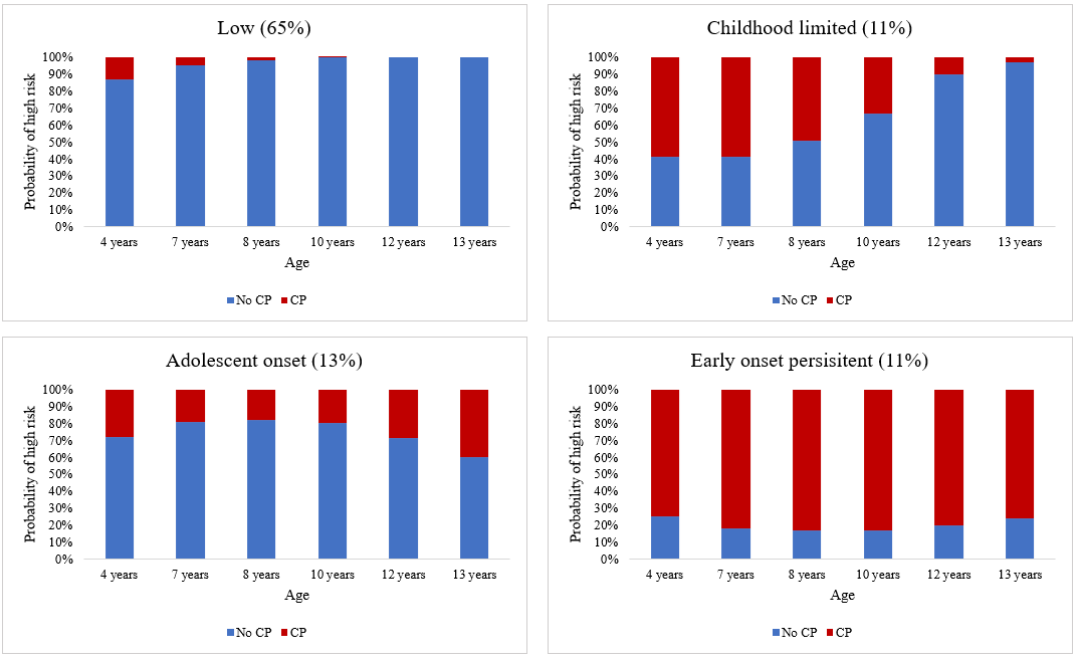

Figure 6.2: Distribution of responses at each timepoint across the four latent classes of conduct problems.

Figure 6.3 displays the proportion of individuals belonging to each conduct problem class for adolescents aged 4-13.

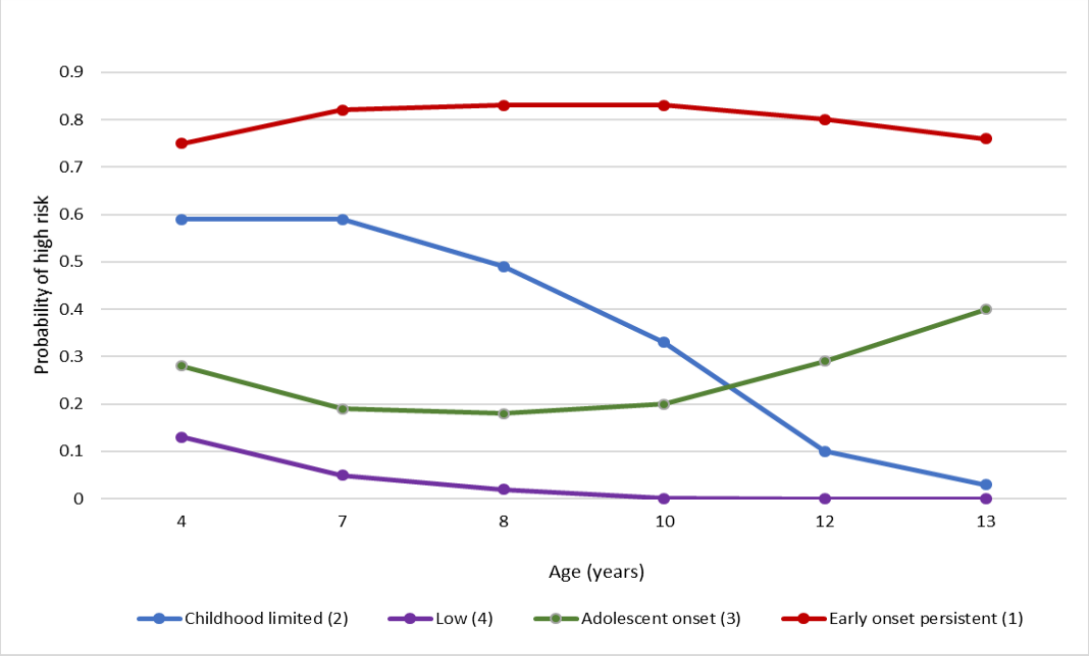

Figure 6.3: Probability of conduct problems present within each latent class across each of the timepoints.

Consuming <1 glass of alcohol per week at 18 weeks gestation was not associated with offspring conduct problems in the adjusted and unadjusted models. Consuming 1 or more glasses of alcohol per week at 18 weeks gestation was associated with offspring childhood limited (OR = 1.48, 95% CI 1.08, 2.05), and early onset persistent conduct problem classes (OR = 1.26, 95% CI 2.63, 5.16) compared to being in the low conduct problems class. After adjustment for potential socioeconomic and maternal behaviours these associations were attenuated (see Table 6.5).

Table 6.5: Associations between maternal PAE and conduct problem trajectories for each latent class

	Unadjusted (*n* = 7095)					Adjusted (*n* = 5309)				
	Low (ref)	CL	AO	EOP	p_a	Low (ref)	CL	AO	EOP	p_a
	OR	OR (95% CI)	OR (95% CI)	OR (95% CI)		OR	OR (95% CI)	OR (95% CI)	OR (95% CI)	
None	(ref)	(ref)	(ref)	(ref)		(ref)	(ref)	(ref)	(ref)	
<1 glass per week	(ref)	1.15 (0.89, 1.50)	0.91 (0.73, 1.13)	1.15 (0.95, 1.41)	0.260	1.00	1.21 (0.88, 1.67)	0.98 (0.76, 1.27)	1.17 (0.92, 1.49)	0.390
1+ glass per week	(ref)	1.48 (1.08, 2.05)	0.84 (0.61, 1.16)	1.26 (0.97, 1.64)	0.020	1.00	1.25 (0.82, 1.91)	0.92 (0.63, 1.34)	1.19 (0.86, 1.64)	0.513

[1]Adjusted for: Socioeconomic position, income, home ownership, marital status, maternal education, parity, maternal tobacco use during 1-3 months of pregnancy, maternal depression 18 weeks gestation. [a]Wald test.

CL: Childhood limited. AO: Adolescent onset. EOP: Early onset persistent.

6.5 Discussion

This chapter investigated how maternal PAE is associated with offspring depressive symptoms and conduct problems over repeated measures in childhood and adolescence. Through latent class analyses I identified four distinct classes of conduct problem trajectories within offspring aged 4-13 in line with previous research (Edwards et al., 2014; Mahedy et al., 2017); low, childhood limited, early onset persistent and adolescent onset. Overall, I found an association between mothers consuming more than one alcoholic drink per week and offspring having conduct problems in childhood. Investigation of the differences between individual classes found that compared to the low conduct class, increased PAE was associated with childhood limited and early onset persistent conduct problems, but not adolescent onset. These associations did not remain after adjustment for potential confounding variables. Within the adjusted models there was almost no difference between the associations shown for each level of alcohol exposure (< 1 glass per week; 1+ glass per week). This suggests that the differences shown in associations between alcohol exposures within the unadjusted models are likely due to confounding influences, and are not causal as there was no dose response patterns shown.

If the associations shown between PAE and offspring conduct problems were causal, the differences in associations being shown within the distinct conduct problem classes for childhood limited and early onset persistent conduct problems, would suggest more proximal influences of PAE compared to distal influences. Previous research has shown differences between conduct problem classes may be due to a range of environmental factors. Higher levels of childhood onset conduct problems have been associated with factors such as poor parenting, compared to adolescent onset problems which were not (Moffitt et al., 2008; Vanderbilt-Adriance et al., 2015). If mothers who consumed alcohol during pregnancy were likely to also consume more alcohol postnatally, this could be linked to a difference in parenting styles which may then influence offspring's behaviour in early childhood.

Previous research has found evidence for an association between parental alcohol use and offspring behavioural problems (D'Onofrio et al., 2007; Disney et al., 2008). However, this may be due to the samples used often consisting of clinical samples including individuals with severe conduct problems, or higher levels of parental alcohol use such as dependence. This is supported by the findings of Mahedy and colleagues (Mahedy et al., 2017), who in line with the analyses from this chapter investigated lower levels of parental alcohol use (yet this time postnatally) and

associations with offspring conduct problems and found limited evidence of an association.

In this chapter I also investigated the influence of maternal PAE on offspring trajectories of depressive symptoms, across repeated measures from offspring aged 4-13. Maternal PAE at 18 weeks gestation was not shown to be associated with offspring depressive symptoms across repeated measures in both unadjusted and adjusted models. These findings are again comparable to Mahedy and colleagues who found insufficient evidence of an association between parental postnatal alcohol use and offspring depressive symptoms. In comparison to Chapters Two and Three that showed evidence of an association between PAE and offspring depression, it may be that any associations are only observed at specific timepoints. However, I feel it is more likely that the differences shown in the current chapter are likely due to methodological reasons such as a small sample size and the use of a less clinical measure of depression. The mean scores of depressive symptoms within ALSPAC at each recorded age were relatively small overall. Maternal PAE was also condensed into two distinct categories of use (<1 glass per week, 1+ glass per week) due to methodological constraints. This is in contrast with previous chapters which used more categories of varying levels of alcohol exposure, and makes it more difficult to identify any trends with varying amounts of alcohol use, even if underpowered.

As discussed in previous chapters the ALSPAC cohort has high attrition, particularly for measures recorded at later ages. As the offspring have aged, higher attrition rates are shown for those who are socially disadvantaged, and potentially could have created a biased sample in those who have remained within the cohort. Due to drop out rates and non-response I therefore did not have complete data for exposures, covariates and outcomes. Previous research has suggested that such attrition does not impact estimates for behavioural problems in ALSPAC, and therefore missing data within the conduct disorder outcome may not have biased the sample (Wolke et al., 2009). However, as I applied FIML to account for the possible bias that may have been present in the available exposure data it is unlikely that bias was present from maternal PAE.

Binary measures of conduct disorder were used within the latent class analyses with each representing not high, and high risk of each class of conduct disorder based on national norms established for 5-10 year olds across gender, in England and Wales. This cut off was used to replicate Barker and Maughan's study which first derived these classes (Barker & Maughan, 2009), as well as Mahedy et al's study which investigated the association between parental postnatal alcohol use and offspring conduct problems (Mahedy et al., 2017). These binary cut offs have been

shown to be strong predictors of conduct disorder (Goodman, 2001), however, binary measures are less sensitive to change compared to continuous models. Using binary cut offs instead of a continuous measure means a loss of information, which in turn means a reduction in power to detect associations (Schmitz, Adams, & Walsh, 2012). The validity of the SDQ measures are also increasingly raised, with previous studies indicating a poor reliability of the conduct problems subscale (Mieloo et al., 2012), see Appendices 6.2 and 6.3. The potentially poor reliability of the conduct problem scale means that by reducing the available information to a binary cut off, we could be introducing more measurement error.

Maternal PAE and offspring conduct problems were both measured by maternal report, which may have also introduced bias, which is particularly likely when exposures and outcomes are reported by the same person (Heron et al., 2013). Differences in findings have previously been shown between self-report and parental report for offspring outcomes (Robinson et al., 2019). Future studies using offspring self-report of conduct problems, and repeated measures of a more clinical measure of depression may be beneficial to investigate influences on offspring behavioural and emotional problems. Future work utilising mediation models to test the influence of various lifetime exposures which were unobserved in the current study, such as parenting styles or puberty, could also help to explain the underlying mechanisms between maternal alcohol exposure and offspring mental health and behavioural problems.

6.6 Chapter summary

This chapter used LCGA and trajectory analyses to investigate the associations between maternal PAE and repeated measures of offspring conduct problems and depressive symptoms. I found little evidence of an association between the amount of alcohol consumed by mothers at 18 weeks gestation and offspring depressive symptoms. There was evidence of an association between maternal PAE (1+ drinks per week) and offspring childhood limited and early onset persistent conduct problems. These findings suggest proximal effects for age of onset of conduct problems in mothers who consume one or more glasses of alcohol per week during pregnancy. However, these associations did not persist after adjustment which suggests the associations shown in the unadjusted models were driven by confounding structures. The difference of these findings compared to Chapters Two and Three which showed associations between PAE and offspring depression, may

be in part due to the measures used in this study, and potentially the use of a non-clinical sample.

Chapter 7 Discussion and future directions

7.1 Summary

When investigating if offspring mental health outcomes are associated with maternal prenatal alcohol use there are many challenges in establishing a true causal effect, such as bias in particular measurements, attrition and confounding influences which may be masking true effects. This thesis applied different methods to explore the potential influence of maternal PAE on offspring mental health during adolescence. These methods included: a systematic review, negative control analyses, a PheWAS, latent class and trajectory analyses. The main findings from this thesis are discussed, as well as potential limitations of the research conducted, and future next steps.

7.1.1 Systematic review of PAE and offspring internalising disorders and conduct disorder

In Chapter Two my aim was to systematically review the already published literature on associations between PAE and offspring internalising disorders, and conduct disorder. This review was exploratory and sought to investigate and describe any patterns of association previously reported for varying types of mental health. Previous literature was found to be less clear on the relationship between PAE and offspring internalising disorders, specifically for low levels of PAE that wasn't focused on FASD. This chapter found evidence of associations of increased risk for mental health problems in offspring prenatally exposed to alcohol, specifically for mental health subtypes of anxiety/depression, total problems and conduct disorder. The exploratory nature of this review aided in highlighting and describing the vast disparity in measurements used between studies. This may perhaps be due to constraints in available data from longitudinal analyses. This review also highlighted how many studies tend to focus on offspring outcomes from earlier ages, often pre-adolescence and within early developmental periods. Only one study within the review investigated PAE and offspring internalising disorders over the age of 18. Further research is therefore required to investigate the influence of PAE on offspring outcomes within late adolescence and early adulthood, to determine if the associations shown in early childhood are also present in adulthood.

7.1.2 Negative control analyses of maternal and partner PAE and offspring depression

In Chapter Three I aimed to extend the findings from my systematic review through conducting a longitudinal study investigating the association between PAE and offspring depression. I sought to expand the previous literature by measuring offspring outcomes at an older age group to ascertain if any associations shown may persist into adulthood. This chapter also aided casual interpretation by applying a negative control methodology which included partner alcohol use during pregnancy. In support of the findings from Chapter Two, I found maternal PAE to be associated (albeit weakly) with offspring depression at age 18 in a dose-response relationship. Partner alcohol use was not shown to be associated with offspring diagnoses of depression at age 18. This would suggest that any associations shown are likely due to intrauterine alcohol exposure from mothers. The associations found between maternal PAE and offspring depression were only weakly attenuated after adjustment for potential confounding influences. This further suggests that the associations shown were due to PAE and not confounding structures.

7.1.3 Parental postnatal alcohol use and offspring mental health

In Chapter Four my aim was to investigate the influence of postnatal parental alcohol use (both maternal and partner) on a range of offspring mental health outcomes in late adolescence. This chapter sought to assess the influence parental alcohol use during a child's upbringing (age 5 years) may have on their mental health in late adolescence. There was no clear evidence that postnatal alcohol use was associated with offspring depression at age 18. Univariate analyses suggested a 'protective' effect of parental alcohol use on offspring total problems. Similar findings were shown between maternal postnatal alcohol use and decreased offspring hyperactivity and emotional problems at age 17. Partner alcohol amount was also associated with decreased conduct problems. Parental binge drinking was not associated with any of the offspring mental health outcomes in univariate analyses, except for decreased emotional symptoms of the SDQ. However, none of these given associations remained after adjustment for socioeconomic confounders and maternal genetic risk for depression. This would suggest that any associations shown in the univariate analyses were being driven by confounding influences. Further investigation into what may be driving these findings were shown in differences between levels of alcohol frequency between socioeconomic factors. Parents from higher socioeconomic backgrounds consumed higher amounts of alcohol at least once a week or drank 1-2 units per day compared to those from lower SES backgrounds. How often parents consumed ≥4 units of alcohol (binge drinking) was fairly stable across

socioeconomic backgrounds. Such findings demonstrate that the apparent 'protective effect' of parents increased alcohol use being associated with reduced mental health problems, is likely due to differences in the confounding structures within the data, and that factors such as socioeconomic status and income are what are actually protective against mental health problems, not parental alcohol use.

7.1.4 The association of alcohol polygenic risk scores on mental health phenotypes: A PheWAS in the Avon Longitudinal Study of Parents and children

In Chapter Five I aimed to build on previous chapters that had shown associations between maternal PAE and offspring mental health by investigating how genetic variants associated with increased alcohol use may affect a wide variety of mental health phenotypes, using a PheWAS design. Within this chapter I validated the PRS for increased alcohol use within the ALSPAC cohort for pregnant mothers, as well as showing an association with increased maternal depression at 32 weeks gestation. I also investigated the intergenerational effects by comparing maternal PRS for increased alcohol consumption with offspring mental health phenotypes and found associations with decreased scores of intellectual ability. It is therefore possible that we are observing intrauterine effects of maternal alcohol use on offspring phenotypes, or further confounding influences of maternal depression. Further analyses sought to test offspring's own PRS for increased alcohol use with their mental health phenotypes and found no effects. However, I also ran analyses to validate if offspring PRS for alcohol use was associated with alcohol use phenotypes and found no effects also. This is likely due to the adolescents drinking behaviours not being fully established and stable, as opposed to the subgroups that the PRS were derived from in the original GWAS (adulthood). In the absence of offspring PRS predicting their alcohol use, I could therefore not explore these potential effects in depth.

7.1.5 PAE and offspring trajectories of depressive symptoms and latent classes of conduct disorder

In Chapter Six my aim was to use longitudinal data of repeated measures to investigate the influence of maternal PAE on trajectories of offspring depressive symptoms and latent classes of offspring conduct problems. Using latent class analyses I identified 4 distinct classes of conduct problem trajectories for adolescents aged 4-13 and tested how PAE may influence these. The classes were low, childhood limited, early onset persistent and adolescent onset conduct problems. By comparing the classes I

showed that compared to the low conduct class, increased PAE was associated with childhood limited and early onset persistent conduct problems, but not adolescent onset. However, as these associations did not remain after adjustment for potential confounders it would suggest that the results shown from the unadjusted models were driven by confounding structures.

Previous literature has investigated trajectories of adolescent depression, but it hasn't been studied in relation to how PAE may be associated with changes in depressive symptoms. I also investigated this in Chapter Six and found little evidence that maternal PAE was associated with trajectories of offspring depressive symptoms in adolescence. This suggests that the slope and intercept for adolescent depression are both not influenced by maternal PAE, and the age of onset and rate of change shown from previous research may be influenced by other mediating factors (such as pubertal timing). However, further replication using larger sample sizes is required.

7.2 Overall findings

This thesis sought to investigate if PAE was associated with offspring mental health in adolescence, particularly around age 18, to assess if any associations shown from previous literature for younger offspring ages as shown in Chapter Two (Easey et al., 2019) may persist into adulthood. The systematic review highlighted the scarcity of research already published which investigates older offspring outcomes, and the following chapters aided in addressing this research question. Vast differences in the measurement of exposures, outcomes, and confounding influences between studies have also made comparisons problematic in evaluating the weight of evidence. Within this thesis, I sought to add to the literature addressing the potential causal effect of PAE, by using negative control analyses of partner alcohol use during pregnancy (Chapter Three). An association was shown only for maternal prenatal alcohol use at 18 weeks gestation and offspring depression at age 18, which was only weakly attenuated after adjustment for potential confounding influences. The use of negative control analyses using partner alcohol use as a potential exposure, showed that associations were only present for maternal alcohol use, and therefore likely due to a maternal intrauterine effect. Previous studies have also often not investigated a dose-response relationship of alcohol exposure and have only investigated offspring exposed and not exposed to alcohol during pregnancy (Bada et al., 2007; Disney et al., 2008; Sayal et al., 2014). Chapter Three found maternal PAE also showed a pattern of a dose-response relationship with increased offspring depression, which is suggestive of a causal relationship of heaviness of amount of alcohol consumed prenatally on offspring depression. Further investigation into the

influence of parental drinking during their offspring's upbringing (Chapter Four) showed no clear evidence of parental postnatal alcohol use having an influence. Instead, offspring mental health was related to parental socio-economic factors, with social advantage in income and employment for example showing protective effects for positive mental health outcomes. Such findings highlight the need for appropriate adjustment for confounding influences when investigating alcohol use, as well as validating that the focus of the thesis should remain on prenatal alcohol exposure. This thesis also sought to investigate multiple mental health outcomes across the phenome (Chapter Five), and across time using repeated measures (Chapter 6) from a longitudinal cohort, to assess the full potential influence of PAE. One outcome was associated with maternal genetic variants for increased alcohol use in Chapter Five, which may be suggesting effects are only present for certain offspring outcomes (decreased intellectual ability). However, this could be in part due to the validity of the mental health outcomes measured, as well as sample size and attrition due to the again later age the outcomes were measured. By investigating the influence of PAE on offspring trajectories and latent classes of mental health (Chapter Six), I was able to assess changes over time and the trends such outcomes may show throughout adolescence. More proximal effects were shown for PAE on conduct problems, as shown by stronger associations for childhood limited conduct problems. However, these associations did not remain after adjustment, suggesting they were due to confounding influences. Investigation of the trajectories of offspring depression following PAE showed no associations. This again may be in part due to measurement of the phenotype, and the low levels of depressive symptoms found in the ALSPAC cohort which could be why different findings were shown from Chapter Three which measured a clinical level diagnoses of depression. The implications of the findings from this thesis suggest an intrauterine effect of a detrimental association between maternal PAE at 18 weeks gestation and offspring mental health.

7.3 Implications and conclusion

In my thesis I found weak evidence for a casual association between maternal PAE and offspring mental health in late adolescence. However, across chapters any associations shown were attenuated or removed entirely after adjustment for potential confounding influences, suggesting that much of the association found may be driven more by factors such as socio-economic characteristics. Such findings show the importance of including demographic attributes as confounding variables when investigating alcohol and mental health, but also highlight potential areas of risk for poorer mental health outcomes. Previous research has also shown much of the associations between PAE and offspring mental health to be driven by confounding

influences such as greater parental income or education for example. My findings are comparable to Kelly and colleagues who found that increased PAE was associated with decreased mental health outcomes in offspring (Kelly et al., 2009). The same patterns were also shown in a later study evidencing increased PAE to be associated with decreased total difficulties as well as higher cognitive scores in offspring (Kelly et al., 2012). However, this apparent 'protective' effect of increased PAE was explained by the authors as not actually being due to alcohol use during pregnancy, but instead due to residual confounding. This is comparable to my findings for Chapter Four, which appeared to show parental postnatal alcohol use to be associated with reduced offspring mental health problems. However, again this apparent 'protective' effect of parental alcohol use was removed after adjustment for confounding variables of socioeconomic characteristics. These findings do not suggest on their own that maternal alcohol use during pregnancy is safe for offspring, or that negative mental health outcomes are driven solely by confounding influences.

A challenge within this area of research is shown in our inability to always appropriately measure FASD. I sought to avoid measuring FAS and FASD within this thesis, due to the heavy focus previous research of PAE had concerning these outcomes. However, I cannot be certain that the analyses conducted within this thesis have not also included individuals with undiagnosed FASDs. This is a challenge of a lack of appropriate diagnostic tools for FASD, of which the aetiology is multifaceted and complex (McQuire et al., 2019). Although I cannot be certain, I have applied various methods to reduce the risk of including individuals with FASD within this thesis. Within the systematic review I excluded any papers that reported using offspring with FAS/FASD, as well as making the majority of the focus for this thesis to be on internalising disorders as opposed to externalising disorders, which are more prevalent in FASD samples. If, however, the findings are actually representative of FASD as an outcome and not offspring mental health separately, this could be indicating that the prevalence of FASD is higher than already believed. Better diagnostic tools for FASD would benefit this research, particularly as FASD does not evidence the same physical attributes as FAS (Jones & Smith, 1973; Jones et al., 1973) which make it easier to diagnose.

The analyses conducted within this thesis have also encountered the challenges often shown in this area of research, which are discussed in greater detail in the limitations section below. Further research is required to investigate the causal effects of PAE on offspring outcomes, particularly for low to moderate amounts of alcohol. A recent systematic review of light alcohol use during pregnancy and offspring outcomes found limited evidence for a causal role (Mamluk et al., 2017). However, the authors

stress that not only is there a paucity of research being conducted within this area, but also the research that has been conducted is also poor in assessing causal inference. This study highlighted that absence of evidence of harm, is not evidence of absence. A review published this year sought to review the literature that has used causal inference methods when investigating PAE and offspring outcomes, finding a detrimental effect of cognitive outcomes (Mamluk et al., 2020). The findings from this thesis adds to the growing body of research that investigates light to moderate maternal alcohol use during pregnancy on offspring outcomes, highlighting the many challenges faced in this area, which add to the problems in inferring the causal nature of effect. The findings of PAE still evidencing associations with mental health outcomes even during late adolescence, would suggest the associations previously seen within the younger developmental ages may indeed persist until early adulthood. Such findings offer support for the UK's Department of Health's recommendations of complete abstinence during pregnancy and for those trying to conceive.

7.4 Limitations of this research

As discussed throughout this thesis each chapter has potential limitations which may have influenced the strength and validity of my findings. The research undertaken in this thesis concerns observational epidemiology, and as such may include the potential problems known to be a part of such epidemiological research. The main challenges I found when investigating PAE and offspring mental health outcomes are discussed below.

7.4.1 Attrition in cohort studies

One of the main limitations faced and widely acknowledged when using longitudinal birth cohorts, such as ALSPAC which was used throughout this thesis, is loss to follow up. As participants were followed for such an extensive period, a high rate of attrition has been shown from the over 15,000 participants who originally enrolled. This could potentially result in selection bias where the individuals who remain in the cohort may be different to those who have dropped out. Selection biases could lead to over estimation of the 'protective effects' previously discussed between low to moderate alcohol use and health outcomes (Naimi et al., 2019; Naimi, Stockwell, Saitz, & Chikritzhs, 2017). As I was focusing on one of the later ages that data was available for at the start of this thesis, it was inevitable that I would encounter high rates of loss to follow up. Within this thesis I tried to account for this, and conducted and compared results from

multiply imputed datasets and complete case datasets where possible, finding little difference between each model.

7.4.2 Confounding influences

As well as selection bias, residual confounding may explain any observed observations due to incomplete adjustment of measured and unmeasured confounders. By using longitudinal population cohorts to conduct research, we are only able to include measures that have been recorded within each study which may mean at times important confounding measures may not be included which could be influencing the findings. As discussed in previous chapters, confounding influences have been previously shown to be associated with alcohol use. Increased alcohol use has often shown a J-shaped relationship with detrimental physical health outcomes, whereby increased alcohol consumption up to a certain point is shown to be protective against cardiovascular problems. However, this is likely not to be due to alcohol use, but instead due to confounding structures as discussed in Chapter Four. Mental health has also shown the same J-shaped curve patterns, with low levels of alcohol use being associated with positive mental health outcomes. For example, decreased risk of offspring problems has been shown for offspring prenatally exposed to light levels of alcohol during pregnancy. Kelly and colleagues (2010) showed offspring born to mothers who consumed low amounts of alcohol during pregnancy had higher cognitive scores. However, after adjustment for various socio-economic factors these associations were attenuated, and the previous 'protective' associations shown were therefore likely not to be due to alcohol use. More recent research suggests there is no level of alcohol consumption that improves health, and more advanced epidemiological methods such as triangulation help to show this (Burton, Zhang, Boa-Amponsem, Mackinnon, & Cole, 2017; Lawlor et al., 2016; Munafò & Davey Smith, 2018).

7.4.3 Self-reported data of exposure

Many of the measures included within each chapter were taken from self-reported data and may therefore suffer from misclassification errors. The underreporting of PAE could attenuate estimates of association between exposures and outcomes (Lawlor et al., 2016). Maternal self-report of PAE is the most common method for assessing exposure amount, often via standardized questionnaires (E. Burns, Gray, & Smith, 2010; Russell et al., 1996). The validity of these questionnaires is often queried as pregnant mothers could underreport their alcohol use for fear of stigma (Durant, Carey, & Schroder, 2002). Concurrent measures of alcohol use during pregnancy have been shown to likely

underestimate fetal exposure (Alvik et al., 2006). An alternative method of obtaining accurate PAE amounts is through using biomarkers of alcohol use. However, it is not always possible to find a suitable marker for alcohol use which is more reliable than self-report. The validity of such measures is currently still questioned with reviews finding insufficient evidence to support objective measures of PAE and found meconium to be more appropriate biomarkers (McQuire et al., 2016). Estimates of PAE from meconium testing has been shown on average to be four times higher than maternal self-reports (Lange, Shield, Koren, Rehm, & Popova, 2014). Meconium testing for PAE also does not appear to be common practice for many of the large cohorts in which longitudinal data can be used to investigate offspring outcomes. As well as mainly being able only to detect binge drinking, it can also only show PAE from the second and third trimester of pregnancy, but not the first trimester, which is when the strongest associations have been shown with offspring outcomes (Chapter 3). In Chapter 5, instead of self-report measures I used genetic variants associated with increased alcohol use as a proxy for PAE. This inclusion of a different measure of PAE helps to test the previous chapter's findings of the influence of increased maternal alcohol use.

7.4.4 Generalisability

Throughout this thesis the ALSPAC cohort was used in each empirical chapter. However, this may mean that the findings from this thesis are not generalisable to the rest of both the UK and the world. Mothers within the ALSPAC cohort are shown to have higher socioeconomic characteristics compared to not only the rest of Avon, but also the rest of Great Britain. Fraser and colleagues showed mothers from the ALSPAC cohort were much more likely to own their property, have access to a car in their household and be married (Fraser et al., 2013). Fraser and colleagues also showed that only 2.2% of the mothers involved were not white, compared to 7.6% of the general population at the time of enrolment. Additionally, this cohort analyses found mothers who remained in the cohort ~18 years after pregnancy were more likely to be from a lower socio-economic background, less likely to have a university degree and were older. Such findings highlight that mothers who have both enrolled and remained in the ALSPAC cohort may have limited generalisability to the UK population. Future research would benefit from the inclusion of additional cohorts which have varying socio-demographic characteristics. Replication of these studies within varying cohorts would allow more certainty for if the findings from this thesis are robust or not.

7.5 Strengths of this research

A strength of this thesis is in the use of triangulation of various methodologies in an attempt to strengthen causal inference for the effect of maternal prenatal alcohol use on offspring mental health in late adolescence. This research question requires observational studies, and we are unable to use ethically use randomised control trials to assess the influence alcohol exposure during pregnancy may have. The strengths of research within this area therefore rely on the methodology used. By using a negative control design of partner alcohol use during pregnancy where there is no reasonable biological explanation, we were able to ascertain if any associations shown were likely to be causal. This study was the first to apply a negative control design to prenatal alcohol exposure on offspring internalising disorders and added to the literature in assessing casual inference whilst attempting to minimising confounding effects.

As discussed in previous chapters, how PAE is measured by self-report varies considerably between studies in previous research. Within the systematic review (Chapter Two), out of the 33 included studies 29 used varying categorical exposure measures. The remaining 4 which used the same measure did so through a binary measure asking if alcohol was consumed during pregnancy (yes/no). This highlights that even if we are able to measure alcohol use appropriately, comparisons between studies to ascertain what level of exposure may be harmful/safe becomes problematic. By using the same exposure measure of PAE within the empirical chapters where possible, I was able to aid in comparison across chapters, and had removed bias that may have been caused through measurement disparity. By not using a binary measure of exposed, not exposed to PAE I was also able to investigate if there was a certain amount of alcohol exposure that may have been harmful to the developing fetus. In particular, this evidenced a linear trend of increased PAE and offspring depression in the negative control chapter, for maternal alcohol use only and not partner alcohol use.

The PheWAS chapter of this thesis added a novel contribution to the literature for multiple reasons. First, by using genetic variants shown to be associated with increased alcohol use, we were able to avoid some of the limitations of self-reported PAE, which as previously discussed can be plagued by measurement error, particularly during pregnancy and when measured retrospectively. Using genetic variants also meant that we were able to limit the influence of potential confounding factors (Davey Smith & Ebrahim, 2004). I was also the first to confirm that these genetic variants for increased alcohol consumption were valid measures for PAE in pregnancy. Such confirmation is advantageous for future research seeking to use genetic markers of alcohol use during pregnancy. Secondly, the PheWAS analyses used very detailed phenotyping of mental health within ALSPAC, for

mental health measures. I measured all mental health phenotypes that were available within ALSPAC, meaning inferences of the influence PAE has on mental health can be made across the whole phenome (of measured phenotypes within the cohort).

7.6 Future directions

Although the framework of a PheWAS (Chapter 5) is similar in design to MR, as discussed, a PheWAS does not empirically test the assumptions required within a MR analysis. The analysis within Chapter 5 was exploratory in nature as it was hypothesis free, and any associations I found need further exploration in an independent sample. Future work applying MR could aid in testing for horizontal pleiotropy and provide true effect sizes. However, using this method is challenging in practice as the ability to appropriately conduct MR is reliant on a suitable instrumental variable, which is something that is problematic in alcohol use. MR was originally developed for health traits (e.g. C-reactive proteins) and therefore applying the method to social and behavioural traits requires additional considerations. Genetic variants associated with social and behavioural traits such as alcohol use are complex and are often highly pleiotropic and therefore generally suffer from weak instrument bias (Pingault et al., 2018). Genetic variants for alcohol use often explain a small proportion of variance for an exposure (Gage et al., 2016), as confirmed in Chapter Five. This is why alternative methods to MR, such as negative control analyses, were applied in Chapter 3.

Within this thesis the focus was to investigate the influence of maternal prenatal alcohol use on offspring mental health, with a focus on internalising problems. In attempts to fully ascertain how much any associations found were due to maternal alcohol use over environmental attributes, partner alcohol use was also investigated in Chapters 3 and 4. However, partner alcohol use was also included as an exposure measure mainly due to it's ability to be used within a suitable negative control analysis in Chapter 3. Future work is required to discover the influence of paternal and partner behaviours during pregnancy on offspring mental health. Much of the research previously conducted within the area of developmental origins of health and disease (DOHaD) has primarily focused on how maternal behaviours during pregnancy may influence offspring outcome. Within published research concerning DOHaD, almost 20 times more studies have been published using terms relating to maternal influences compared to paternal (Sharp et al., 2018). Sharp and colleagues highlight the potential reasons why this imbalance may occur, often due to maternal data being more readily available, but largely due to long held assumptions of the causal role of maternal behaviours. Further investigation of articles published in the Journal of the Developmental Origins of Health and Disease in

the 10 years since it began, showed that 61% studied maternal exposures in isolation, 6% studied both parents and less than 1% studied paternal exposures in isolation (Sharp et al., 2019). Even more interestingly these patterns of findings were replicated in animal models, where there are no constraints in availability of data collection by gender. Such findings could also possibly be highlighting a publication bias. Sharp and colleagues' findings evidence a clear disparity of research including father and partner exposures, however these studies did not investigate alcohol exposures in isolation. Future work is required investigating the causal effect of not only maternal health behaviours (such as alcohol use) on offspring outcomes, but paternal also, utilising available data on preconception parental health.

7.7 Further implications

The research conducted within this thesis demonstrates the poor quality of evidence within the area of PAE and offspring mental health, often due to methodological constraints. The paucity of evidence also previously conducted on how light to moderate alcohol exposures during pregnancy may be associated with offspring internalising disorders, make conclusions of its potential harm challenging. Indeed, even the recent updates to the DoH's recommendations for abstinence during pregnancy, are given under the precautionary principle due to the absence of solid evidence for harm. More recent research has emphasised how absence of evidence for harm does not give evidence of absence (Mamluk et al., 2017). The findings from this thesis of light to moderate PAE being associated with detrimental offspring mental health outcomes, adds to the body of evidence within this area and supports the DoH's recommendation of abstinence. The global prevalence of maternal alcohol use during pregnancy was 9.8% indicating that alcohol use in pregnancy is still widespread (Popova, Lange, Probst, Gmel, et al., 2017). Further work evidencing the harmful effects of consuming alcohol during pregnancy could therefore be beneficial in advising policy change and public health interventions.

7.8 Conclusions

The aim of this thesis was to examine the influence of maternal PAE during pregnancy on offspring mental health in late adolescence. This meant I needed to use later measures (from older ages) of childhood mental health problems, however this is often challenging as it meant the data used often had higher rates of attrition and that individuals who did remain within the ALSPAC cohort will have been exposed to varying life factors throughout childhood and adolescence which may have influenced any findings. Overall, my thesis utilised different methodologies to add to the existing

literature. A systematic review highlighted the vast differences between studies measurements assessing PAE and offspring internalising disorders, meaning comparisons across studies is problematic. Further investigation using the ALSPAC cohort, showed evidence for an association between maternal PAE and offspring depression, however, these associations do not go as far as to infer causality. Further work using methods to aid in triangulation would help to assess the causal effect of PAE on offspring mental health. Being able to understand the true causal nature of intrauterine alcohol exposure on offspring mental health is vital to learn how to reduce potential harm. Postnatal alcohol use was found to be associated with offspring mental health, but this seemed heavily driven by confounding influences over alcohol use. Further attempts to assess the causal nature of PAE by investigating the effect of genetic variants for increased alcohol use on mental health phenotypes was limited by a weak instrument for this question. However, findings did suggest a potential intrauterine effect of maternal PAE on offspring outcomes. As well as describing how PAE may affect mental health across a range of phenotypes, how PAE may affect specific phenotypes across continued timepoints was also investigated. This found increased PAE was associated with childhood conduct problems in unadjusted models, but not offspring depression. However, these associations appeared to be driven by confounding structure as any association disappeared within adjusted models. Overall, the findings from this thesis would support abstinence from alcohol use during pregnancy until we are able to causally investigate the levels of harm further.

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Appendices

Appendix 2.1: Studies excluded at full text stage of the systematic review

Author	Year	Reason for exclusion
Alvik et al	2011	Exposures not relevant
Baglot et al	2016	Conference abstract/poster only
Barbier et al	2008	Conference abstract/poster only
Bhatara et al	2006	Exposures not relevant
Chasnoff et al	2015	Exposures not relevant
Chen	2012	Outcomes not relevant
Coles et al	1997	FAS sample
Delaney-Black et al	1998	Outcomes not relevant
Delaney-Black et al	2000	Outcomes not relevant
Enoch et al	2016	Outcomes not relevant
Hanna et al	1997	Outcomes not relevant
Howell et al	2006	Outcomes not relevant
Infante et al	2015	Outcomes not relevant
Skarpness et al	2012	Outcomes not relevant
Knopik et al	2005	Conference abstract/poster only
Kukla et al	2008	Not English language publication
Mick et al	2002	Outcomes not relevant
Motz et al	2013	Commentary
O'Connor et al	2002	Outcomes not relevant
O'Connor et al	2002	Exposure not relevant
Piper et al	2014	Exposure not relevant
Rasmussen et al	2011	Exposure not relevant
Rettew	2008	Commentary
Rodriguez et al	2009	Outcomes not relevant
Salom et al	2014	Exposures not relevant
Sato et al	2008	Conference abstract/poster
Sayal et al	2007	Commentary
Sciberras et al	2011	Outcomes not relevant

Smith	2016	Commentary
Sood et al	2002	Conference abstract/poster
Way et al	2012	FAS group
Willford et al	2006	Outcome not relevant

FAS: Fetal alcohol syndrome

Appendix 3.1: Associations between maternal binge drinking at 32 weeks and offspring depression (CIS-R) at age 18, imputed data

	Unadjusted		Adjusted[1]		Adjusted[2]	
n = 13480	OR (CI)	*P*	OR (CI)	*P*	OR (CI)	*P*
None	1.00 (ref)	0.564[a]	1.00 (ref)	0.735[a]	1.00 (ref)	0.724[a]
1-2 days	1.09 (0.77-1.55)		1.01 (0.70-1.45)		0.96 (0.66-1.39)	
3-4 days	1.50 (0.93-2.42)		1.38 (0.84-2.27)		1.27 (0.76-2.12)	
5-10 days	0.86 (0.35-2.14)		0.80 (0.31-2.03)		0.69 (0.26-1.81)	
>10 days	0.90 (0.32-2.57)		0.81 (0.28-2.36)		0.69 (0.23-2.03)	
Linear trend	1.05 (0.91-1.22)	0.491	1.02 (0.87-1.19)	0.846	0.97 (0.83-1.15)	0.742

Model[1] adjusted initially for social class, income, home ownership, marital status, maternal education, gender and parity. Model[2] further adjusted for tobacco use during 1-3 months of pregnancy, illicit drug use during 1-3 months of pregnancy and maternal depression at 18 weeks gestation

[a]Wald test

Appendix 3.2: Associations between maternal binge drinking at 32 weeks gestation and offspring depression (CIS-R) at age 24, imputed data

	Unadjusted		Adjusted[1]		Adjusted[2]	
n = 13480	OR (CI)	*P*	OR (CI)	*P*	OR (CI)	*P*
None	1.00 (ref)	0.0004[a]	1.00 (ref)	0.003[a]	1.00 (ref)	0.008[a]
1-2 days	1.47 (1.05-2.08)		1.34 (0.94-1.91)		1.29 (0.90-1.84)	
3-4 days	1.01 (0.52-1.99)		0.89 (0.45-1.78)		0.84 (0.42-1.68)	
5-10 days	1.28 (0.58-2.81)		1.12 (0.49-2.57)		1.05 (0.46-2.41)	
>10 days	4.66 (2.12-10.27)		4.34 (1.89-9.98)		3.94 (1.69-9.20)	
Linear trend	1.28 (1.12-1.47)	0.001	1.23 (1.06-1.43)	0.006	1.20 (1.03-1.40)	0.019

Model[1] adjusted initially for social class, income, home ownership, marital status, maternal education, gender and parity.
Model[2] further adjusted for tobacco use during 1-3 months of pregnancy, illicit drug use during 1-3 months of pregnancy and maternal depression at 18 weeks gestation
[a]Wald test

Appendix 3.3: Associations between maternal alcohol frequency at 18 weeks gestation and offspring depression (CIS-R) at age 18, full sample

	Unadjusted *n* = 4191		Adjusted[1] *n* = 3203		Adjusted[2] *n* = 3027		Adjusted[3] *n* = 2566	
	OR (CI)	*P*	OR (CI)	*P*	OR (CI)	*P*	OR (CI)	*P*
Never	1.00 (ref)	0.060[b]	1.00 (ref)	0.640[b]	1.00 (ref)	0.742[b]	1.00 (ref)	0.734[b]
<1 glass per week	1.16 (0.91-1.49)		1.01 (0.76-1.36)		0.92 (0.68-1.25)		0.89 (0.64-1.25)	
1+ glass per week	1.12 (0.79-1.60)		1.09 (0.73-1.65)		0.97 (0.63-1.49)		0.96 (0.59-1.56)	
1-2 glasses per day	1.86 (0.87-3.98)		1.57 (0.64-3.86)		1.45 (0.57-3.67)		1.82 (0.56-5.86)	
3+ glasses per day	5.78 (1.76-19.02)		3.21(0.63-16.44)		2.33 (0.44-12.49)		2.19 (0.23-20.84)	
Linear trend	1.16 (1.01-1.34)	0.035	1.09 (0.92-1.30)	0.297	1.03 (0.86-1.23)	0.742	1.01 (0.82-1.25)	0.895

Model[1] adjusted initially for social class, income, home ownership, marital status, maternal education, gender and parity.
Model[2] further adjusted for tobacco use during 1-3 months of pregnancy, illicit drug use during 1-3 months of pregnancy and maternal depression at 18 weeks gestation.
Model[3] further adjusted for partner alcohol consumption during pregnancy.
[b]Omnibus p-value

Appendix 3.4: Associations between maternal binge drinking at 18 weeks gestation and offspring depression (CIS-R) at age 18, full sample

	Unadjusted *n* = 4169		Adjusted[1] *n* = 3196		Adjusted[2] *n* = 3021		Adjusted[3] *n* = 2580	
	OR (CI)	*P*	OR (CI)	*P*	OR (CI)	*P*	OR (CI)	*P*
none	1.00 (ref)	0.123[b]	1.00 (ref)	0.161[b]	1.00 (ref)	0.340[b]	1.00 (ref)	0.352[b]
1-2 days	1.35 (0.92-2.00)		1.36 (0.86-2.14)		1.16 (0.71-1.90)		1.29 (0.76-2.20)	
3-4 days	1.88 (1.11-3.18)		1.81 (0.96-3.42)		1.64 (0.83-3.25)		1.58 (0.74-3.37)	
5-10 days	1.29 (0.55-3.02)		1.52 (0.63-3.67)		1.39 (0.57-3.38)		1.72 (0.68-4.35)	
>10 days	1.39 (0.63-3.06)		0.47 (0.11-1.96)		0.41 (0.09-1.81)		0.37 (0.49-2.82)	
Linear trend	1.16 (1.02-1.32)	0.026	1.08 (0.91-1.27)	0.373	1.03 (0.86-1.23)	0.750	1.08 (0.89-1.32)	0.435

Model[1] adjusted initially for social class, income, home ownership, marital status, maternal education, gender and parity. Model[2] further adjusted for tobacco use during 1-3 months of pregnancy, illicit drug use during 1-3 months of pregnancy and maternal depression at 18 weeks gestation. Model[3] further adjusted for partner alcohol consumption during pregnancy.

[b]Omnibus p-value

Appendix 3.5: Associations between maternal binge drinking at 32 weeks gestation and offspring depression (CIS-R) at age 18, full sample

	Unadjusted *n*= 2932		Adjusted[1] *n*=2316		Adjusted[2] *n*= 2213	
	OR (CI)	*P*	OR (CI)	*P*	OR (CI)	*P*
none	1.00 (ref)	0.039[b]	1.00	0.115[b]	1.00 (ref)	0.426[b]
1-2 days	1.07 (0.66-1.73)		0.95 (0.54-1.66)		0.83 (0.45-1.51)	
3-4 days	2.68 (1.47-4.85)		2.32 (1.16-4.65)		1.73 (0.81-3.74)	
5-10 days	0.52 (0.13-2.17)		0.58 (0.14-2.44)		0.56 (0.13-2.40)	
>10 days	1.48 (0.44-4.93)		2.62 (0.72-9.49)		1.84 (0.49-6.96)	
Linear trend	1.14 (0.95-1.36)	0.150	1.16 (0.94-1.42)	0.172	1.06 (0.84-1.32)	0.628

Model[1] adjusted initially for social class, income, home ownership, marital status, maternal education, gender and parity.
Model[2] further adjusted for tobacco use during 1-3 months of pregnancy, illicit drug use during 1-3 months of pregnancy and maternal depression at 18 weeks gestation.
[b]Omnibus p-value

Appendix 3.6: Associations between maternal binge drinking at 18 weeks gestation and offspring depression (CIS-R) at age 18, complete case

	Unadjusted		Adjusted[1]		Adjusted[2]	
n = 3021	OR (CI)	*P*	OR (CI)	*P*	OR (CI)	*P*
none	1.00 (ref)	0.118[b]	1.00 (ref)	0.256[b]	1.00 (ref)	0.340[b]
1-2 days	1.38 (0.86-2.21)		1.24 (0.76-2.01)		1.16 (0.71-1.90)	
3-4 days	2.09 (1.09-4.02)		1.90 (0.97-3.70)		1.64 (0.83-3.25)	
5-10 days	1.79 (0.75-4.25)		1.51 (0.62-3.66)		1.39 (0.57-3.38)	
>10 days	0.64 (0.15-2.67)		0.53 (0.13-2.26)		0.41 (0.09-1.81)	
Linear trend	1.14 (0.97-1.35)	0.111	1.08 (0.91-1.29)	0.354	1.03 (0.86-1.23)	0.750

Model[1] adjusted initially for social class, income, home ownership, marital status, maternal education, gender and parity.
Model[2] further adjusted for tobacco use during 1-3 months of pregnancy, illicit drug use during 1-3 months of pregnancy and maternal depression at 18 weeks gestation.
[b]Omnibus p-value

Appendix 3.7: Associations between maternal binge drinking at 32 gestation and offspring depression (CIS-R) at age 18, complete case

	Unadjusted		Adjusted[1]		Adjusted[2]	
n = 2213	OR (CI)	*P*	OR (CI)	*P*	OR (CI)	*P*
0. none	1.00 (ref)	0.139[b]	1.00 (ref)	0.298[b]	1.00 (ref)	0.426[b]
1. 1-2 days	1.06 (0.60-1.88)		0.95 (0.53-1.70)		0.83 (0.45-1.51)	
2. 3-4 days	2.47 (1.19-5.13)		1.96 (0.92-4.16)		1.73 (0.81-3.74)	
3. 5-10 days	0.67 (0.16-2.80)		0.63 (0.15-2.67)		0.56 (0.13-2.40)	
4. >10 days	2.57 (0.74-8.93)		2.53 (0.70-9.23)		1.84 (0.49-6.96)	
Linear trend	1.19 (0.96-1.46)	0.105	1.13 (0.91-1.41)	0.257	1.06 (0.84-1.32)	0.628

Model[1] adjusted initially for social class, income, home ownership, marital status, maternal education, gender and parity.
Model[2] further adjusted for tobacco use during 1-3 months of pregnancy, illicit drug use during 1-3 months of pregnancy and maternal depression at 18 weeks gestation.
[b]Omnibus p-value

Appendix 3.8: Associations between partner alcohol frequency at 18 weeks gestation and offspring depression (CIS-R) at age 18, full sample

	Unadjusted *n* = 3416		Adjusted[1] *n* = 2708		Adjusted[2] *n* = 2572		Adjusted[3] *n* = 2566	
	OR (CI)	*P*	OR (CI)	*P*	OR (CI)	*P*	OR (CI)	*P*
Never	1.00 (ref)	0.057[b]	1.00 (ref)	0.398[b]	1.00 (ref)	0.338[b]	1.00 (ref)	0.339[b]
<1 glass per week	1.41 (0.69-2.90)		1.42 (0.59-3.43)		1.63 (0.62-4.27)		1.64 (0.62-4.30)	
1+ glass per week	0.96 (0.48-1.94)		1.06 (0.45-2.53)		1.27 (0.49-3.27)		1.26 (0.49-3.26)	
1-2 glasses per day	0.80 (0.37-1.71)		0.91 (0.36-2.31)		0.97 (0.35-2.68)		0.95 (0.34-2.67)	
3+ glasses per day	1.10 (0.45-2.65)		1.24 (0.43-3.55)		1.24 (0.39-3.89)		1.20 (0.38-3.82)	
Linear trend	0.86 (0.74-1.00)	0.045	0.90 (0.75-1.07)	0.245	0.88 (0.73-1.05)	0.163	0.88 (0.73-1.06)	0.172

Model[1] adjusted initially for social class, income, home ownership, marital status, maternal education, gender and parity.
Model[2] further adjusted for tobacco use during 1-3 months of pregnancy, illicit drug use during 1-3 months of pregnancy and maternal depression at 18 weeks gestation.
Model[3] further adjusted for partner alcohol consumption during pregnancy.
[b]Omnibus p-value

Appendix 3.9: Associations between partner binge drinking at 18 weeks gestation and offspring depression (CIS-R) at age 18, full sample

	Unadjusted *n* = 3440		Adjusted[1] *n* = 2723		Adjusted[2] *n* = 2586		Adjusted[3] *n* = 2580	
	OR (CI)	*P*	OR (CI)	*P*	OR (CI)	*P*	OR (CI)	*P*
None	1.00 (ref)	0.657[b]	1.00 (ref)	0.329[b]	1.00 (ref)	0.304[b]	1.00 (ref)	0.289[b]
1-2 days	1.01 (0.67-1.53)		1.06 (0.65-1.74)		1.04 (0.63-1.76)		1.04 (0.62-1.75)	
3-4 days	0.87 (0.57-1.32)		0.97 (0.59-1.58)		0.98 (0.59-1.62)		0.97 (0.58-1.61)	
5-10 days	1.06 (0.73-1.54)		1.27 (0.81-1.99)		1.19 (0.75-1.89)		1.17 (0.73-1.86)	
>10 days	0.82 (0.53-1.25)		0.80 (0.47-1.33)		0.71 (0.42-1.22)		0.69 (0.40-1.20)	
Linear trend	0.97 (0.89-1.07)	0.544	0.99 (0.89-1.10)	0.797	0.96 ((0.86-1.07)	0.465	0.95 (0.85-1.07)	0.413

Model[1] adjusted initially for social class, income, home ownership, marital status, maternal education, gender and parity.
Model[2] further adjusted for tobacco use during 1-3 months of pregnancy, illicit drug use during 1-3 months of pregnancy and maternal depression at 18 weeks gestation.
Model[3] further adjusted for partner alcohol consumption during pregnancy.
[b]Omnibus p-value

Appendix 3.10: Associations between partner alcohol frequency at 18 weeks gestation and offspring depression (CIS-R) at age 18, complete case

	Unadjusted		Adjusted[1]		Adjusted[2]	
n=2572	OR (CI)	*P*	OR (CI)	*P*	OR (CI)	*P*
Never	1.00 (ref)	0.128[b]	1.00 (ref)	0.356[b]	1.00 (ref)	0.338[b]
<1 glass per week	1.62 (0.63-4.17)		1.61 (0.62-4.20)		1.63 (0.62-4.27)	
1+ glass per week	1.18 (0.47-2.99)		1.24 (0.48-3.17)		1.27 (0.49-3.27)	
1-2 glasses per day	0.86 (0.32-2.34)		0.97 (0.35-2.69)		0.97 (0.35-2.68)	
3+ glasses per day	1.50 (0.49-4.56)		1.36 (0.44-4.24)		1.24 (0.39-3.89)	
Linear trend	0.88 (0.73-1.05)	0.158	0.90 (0.75-1.08)	0.241	0.88 (0.73-1.05)	0.163

Model[1] adjusted initially for social class, income, home ownership, marital status, maternal education, gender and parity.
Model[2] further adjusted for tobacco use during 1-3 months of pregnancy, illicit drug use during 1-3 months of pregnancy and maternal depression at 18 weeks gestation.
[b]Omnibus p-value

Appendix 3.11: Associations between partner binge drinking at 18 weeks gestation and offspring depression (CIS-R) at age 18, complete case

	Unadjusted		Adjusted[1]		Adjusted[2]	
n=2586	OR (CI)	*P*	OR (CI)	*P*	OR (CI)	*P*
None	1.00 (ref)	0.515[b]	1.00 (ref)	0.388[b]	1.00 (ref)	0.304[b]
1-2 days	1.08 (0.65-1.78)		1.08 (0.64-1.80)		1.05 (0.62-1.75)	
3-4 days	1.01 (0.61-1.66)		1.00 (0.60-1.65)		0.97 (0.58-1.62)	
5-10 days	1.25 (0.80-1.97)		1.23 (0.77-1.95)		1.19 (0.75-1.89)	
>10 days	0.84 (0.50-1.41)		0.77 (0.45-1.31)		0.71 (0.42-1.22)	
Linear trend	0.99 (0.89-1.10)	0.879	0.97 (0.87-1.09)	0.630	0.96 (0.86-1.07)	0.465

Model[1] adjusted initially for social class, income, home ownership, marital status, maternal education, gender and parity.
Model[2] further adjusted for tobacco use during 1-3 months of pregnancy, illicit drug use during 1-3 months of pregnancy and maternal depression at 18 weeks gestation.
[b]Omnibus p-value

Appendix 4.1: Associations between maternal and partner alcohol frequency at 5 years and offspring depression (CIS-R) at age 18

		Unadjusted (n = 3730)		Adjusted[1] (n = 2824)		Adjusted[2] (n = 2073)	
		OR (CI)	*P*	OR (CI)	*P*	OR (CI)	*P*
Mothers	Never	1.00 (ref)	0.781[a]	1.00 (ref)	0.979[a]	1.00 (ref)	0.786[a]
	<Once a week	1.09 (0.66, 1.80)		0.97 (0.54, 1.75)		0.18 (-0.95, 1.30)	
	At least once a week	0.98 (0.59, 1.63)		0.99 (0.55, 1.80)		0.11 (-1.02, 1.24)	
	1-2 units every day	0.98 (0.56, 1.71)		0.89 (0.45, 1.73)		0.25 (-0.99, 1.49)	
	At least 3 glasses pd	1.60 (0.64, 4.01)		1.20 (0.40, 3.62)		1.09 (-1.18, 3.36)	
	Linear trend	0.99 (0.86, 1.14)	0.899	0.99 (0.83, 1.17)	0.895		
		Unadjusted (n = 2912)		Adjusted[1] (n = 1751)		Adjusted[2] (n = 1304)	
Fathers	Never	1.00 (ref)	0.113[a]	1.00 (ref)	0.666[a]	1.00 (ref)	0.816[a]
	<Once a week	5.79 (0.78, 42.94)		3.23 (0.43, 24.51)		0.84 (-1.35, 3.03)	
	At least once a week	4.45 (0.61, 32.66)		2.93 (0.39, 1.22)		0.25 (-1.87, 2.37)	
	1-2 units every day	3.66 (0.49, 27.19)		2.49 (0.33, 18.90)		0.48 (-1.68, 2.64)	
	At least 3 glasses pd	4.94 (0.64, 38.38)		2.69 (0.33, 21.78)		0.11 (-2.30, 2.45)	
	Linear trend	0.95 (0.80, 1.13)	0.578	0.96 (0.78, 1.18)	0.712	0.97 (0.76, 1.24)	0.803

Model [1]: socioeconomic position, income, home ownership, marital status, maternal education, gender, parity. Model [2]: socioeconomic position, income, home ownership, marital status, maternal education, gender, parity, maternal tobacco use during 1-3 months of pregnancy, maternal illicit drug use during 1-3 months of pregnancy, maternal depression 18 weeks gestation, maternal PRS for major depressive disorder. [b]Omnibus p-value

Appendix 4.2: Associations between maternal and partner alcohol frequency at 5 years and offspring conduct problems (SDQ) at age 17

		Unadjusted (n = 4967)		Adjusted[1] (n = 3803)		Adjusted[2] (n = 2700)	
		Coef (CI)	*P*	Coef (CI)	*P*	Coef (CI)	*P*
Mothers	Never	(ref)	0.331[a]	(ref)	0.703[a]	(ref)	0.603[a]
	<Once a week	-0.03 (-0.18, 0.13)		0.01 (-0.17, 0.19)		-0.009 (-0.23, 0.22)	
	At least once a week	-0.11 (-0.26, 0.04)		-0.03 (-0.21, 0.15)		-0.05 (-0.28, 0.17)	
	1-2 units every day	-0.08 (-0.24, 0.09)		0.03 (-0.16, 0.23)		0.06 (-0.19, 0.30)	
	At least 3 glasses pd	-0.12 (-0.44, 0.19)		-0.15 (-0.51, 0.21)		-0.16 (0.59, 0.28)	
	Linear trend	-0.04 (-0.08, 0.007)	0.104	-0.007 (-0.06, 0.04)	0.779	0.003 (-0.06, 0.06)	0.922
		Unadjusted (n = 2919)		Adjusted[1] (n = 2330)		Adjusted[2] (n = 1696)	
Fathers	Never	1.00 (ref)	0.054[a]	1.00 (ref)	0.480[a]	1.00 (ref)	0.606[a]
	<Once a week	0.15 (-0.17, 0.48)		0.09 (-0.27, 0.45)		-0.17 (-0.49, 0.45)	
	At least once a week	-0.03 (-0.35, 0.28)		0.02 (-0.33, 0.37)		-0.05 (-0.51, 0.41)	
	1-2 units every day	0.02 (-0.30, 0.33)		0.08 (-0.27, 0.44)		0.06 (-0.40, 0.53)	
	At least 3 glasses pd	-0.09 (-0.44, 0.25)		-0.08 (-0.47, 0.30)		-0.10 (-0.06, 0.40)	
	Linear trend	-0.05 (-0.10, -0.0002)	0.049	-0.02 (-0.08, 0.04)	0.481	0.007 (-0.06, 0.08)	0.854

Model [1]: socioeconomic position, income, home ownership, marital status, maternal education, gender, parity. Model [2]: socioeconomic position, income, home ownership, marital status, maternal education, gender, parity, maternal tobacco use during 1-3 months of pregnancy, maternal illicit drug use during 1-3 months of pregnancy, maternal depression 18 weeks gestation, maternal PRS for major depressive disorder. [b]Omnibus p-value

Appendix 4.3: Associations between maternal and partner alcohol frequency at 5 years and offspring hyperactivity (SDQ) at age 17

	Unadjusted (*n* = 2919)		Adjusted[1] (*n* = 2332)		Adjusted[2] (*n* = 1698)	
	Coef (CI)	P	Coef (CI)	P	Coef (CI)	P
Never	(ref)	0.310[b]	(ref)	0.822[b]	(ref)	0.366[b]
<1 glass per week	-0.26 (-0.76, 0.25)		-0.33 (-0.88, 0.22)		-0.63 (-1.33, 0.06)	
1+ glass per week	-0.35 (-0.84, 0.14)		-0.27 (-0.80, 0.27)		-0.47 (-1.15, 0.21)	
1-2 glasses per day	-0.43 (-0.93, 0.07)		-0.30 (-0.84, 0.24)		-0.48 (-1.16, 0.21)	
3+ glasses per day	-0.28 (-0.81, 0.26)		-0.27 (-0.85, 0.31)		-0.37 (-1.10, 0.36)	
Linear trend	-0.05 (-0.13, 0.03)	0.192	-0.008 (-0.10, 0.08)	0.862	0.03 (-0.07, 0.14)	0.523

Model [1]: socioeconomic position, income, home ownership, marital status, maternal education, gender, parity. Model [2]: socioeconomic position, income, home ownership, marital status, maternal education, gender, parity, maternal tobacco use during 1-3 months of pregnancy, maternal illicit drug use during 1-3 months of pregnancy, maternal depression 18 weeks gestation, maternal PRS for major depressive disorder. [b]Omnibus p-value

Appendix 4.4: Associations between maternal and partner binge drinking at 5 years and offspring depression (CIS-R) at age 18

		Unadjusted (n = 3704)		Adjusted[1] (n = 2813)		Adjusted[2] (n = 2073)	
		OR (CI)	*P*	OR (CI)	*P*	OR (CI)	*P*
Mothers	None	1.00 (ref)	0.548[a]	1.00 (ref)	0.963[a]	1.00 (ref)	0.848[a]
	1-2 days	1.21 (0.90, 1.63)		1.12 (0.79, 1.61)		0.77 (0.40, 1.49)	
	3-4 days	1.01 (0.69, 1.49)		0.95 (0.61, 1.50)		0.83 (0.43, 1.62)	
	5-10 days	1.22 (0.81, 1.83)		1.02 (0.63, 1.66)		0.65 (0.30, 1.43)	
	>10 days	1.38 (0.84, 2.26)		1.02 (0.54, 1.93)		1.16 (0.32, 4.18)	
	Linear trend	1.06 (0.97, 1.17)	0.217	1.00 (0.89, 1.12)	0.987	0.96 (0.78, 1.76)	0.667
		Unadjusted (n = 2186)		Adjusted[1] (n = 1747)		Adjusted[2] (n = 1303)	
Fathers	None	1.00 (ref)	0.096[a]	1.00 (ref)	0.439[a]	1.00 (ref)	0.463[a]
	1-2 days	2.08 (1.14, 3.80)		1.66 (0.85, 3.23)		1.88 (0.24, 14.90)	
	3-4 days	1.66 (0.90, 3.08)		1.29 (0.64, 2.62)		2.05 (0.27, 15.75)	
	5-10 days	1.78 (0.98, 3.22)		1.40 (0.72, 2.73)		1.98 (0.25, 15.55)	
	>10 days	1.27 (0.66,2.42)		1.01 (0.49, 2.07)		1.26 (0.14, 11.53)	
	Linear trend	1.01 (0.89, 1.14)	0.911	0.97 (0.84, 1.12)	0.671	0.97 (0.76, 1.24)	0.803

Model [1]: socioeconomic position, income, home ownership, marital status, maternal education, gender, parity. Model [2]: socioeconomic position, income, home ownership, marital status, maternal education, gender, parity, maternal tobacco use during 1-3 months of pregnancy, maternal illicit drug use during 1-3 months of pregnancy, maternal depression 18 weeks gestation, maternal PRS for major depressive disorder. [b]Omnibus p-value

Appendix 4.5: Associations between maternal and partner binge drinking at 5 years and offspring conduct problems (SDQ) at age 17

		Unadjusted (n = 4929)		Adjusted[1] (n = 3785)		Adjusted[2] (n = 2691)	
		Coef (CI)	*P*	Coef (CI)	*P*	Coef (CI)	*P*
Mothers	None	(ref)	0.100[a]	(ref)	0.328[a]	(ref)	0.201[a]
	1-2 days	0.10 (0.01, 0.20)		0.08 (-0.03, 0.18)		0.07 (-0.06, 0.20)	
	3-4 days	0.14 (0.02, 0.25)		0.12 (-0.008, 0.25)		0.15 (-0.01, 0.31)	
	5-10 days	0.08 (-0.05, 0.21)		0.05 (-0.10, 0.19)		0.11 (-0.07, 0.28)	
	>10 days	0.06 (-0.11, 0.23)		0.11 (-0.08, 0.29)		0.17 (-0.07, 0.40)	
	Linear trend	0.03 (-0.004, 0.06)	0.088	0.03 (-0.008, 0.06)	0.135	0.04 (0.002, 0.09)	0.042
		Unadjusted (n = 2907)		Adjusted[1] (n = 2323)		Adjusted[2] (n = 1691)	
Fathers	None	1.00 (ref)	0.438[a]	1.00 (ref)	0.397[a]	1.00 (ref)	0.281[a]
	1-2 days	0.04 (-0.13, 0.20)		-0.03 (-0.22, 0.15)		0.07 (-0.06, 0.20)	
	3-4 days	0.08 (-0.08, 0.25)		0.09 (-0.10, 0.28)		0.15 (-0.01, 0.31)	
	5-10 days	-0.03 (-0.19, 0.12)		-0.04 (-0.22, 0.13)		0.11 (-0.07, 0.28)	
	>10 days	0.09 (-0.08, 0.25)		0.07 (-0.11, 0.25)		0.17 (-0.07, 0.40)	
	Linear trend	0.009 (-0.03, 0.04)	0.636	0.01 (-0.03, 0.05)	0.544	0.03 (-0.02, 0.08)	0.269

Model [1]: socioeconomic position, income, home ownership, marital status, maternal education, gender, parity. Model [2]: socioeconomic position, income, home ownership, marital status, maternal education, gender, parity, maternal tobacco use during 1-3 months of pregnancy, maternal illicit drug use during 1-3 months of pregnancy, maternal depression 18 weeks gestation, maternal PRS for major depressive disorder. [b]Omnibus p-value

Appendix 4.6: Associations between maternal and partner binge drinking at 5 years and offspring total problems (SDQ) at age 17

		Unadjusted (n = 4927)		Adjusted[1] (n = 3786)		Adjusted[2] (n = 2691)	
		Coef (CI)	P	Coef (CI)	P	Coef (CI)	P
Mothers	None	(ref)	0.102[a]	(ref)	0.248[a]	(ref)	0.118[a]
	1-2 days	0.12 (-0.02, 0.27)		0.09 (-0.07, 0.25)		0.08 (-0.11, 0.27)	
	3-4 days	0.24 (0.06, 0.42)		0.23 (0.03, 0.43)		0.32 (0.08, 0.56)	
	5-10 days	0.09 (-0.11, 0.29)		0.10 (-0.12, 0.32)		0.16 (-0.11, 0.42)	
	>10 days	0.15 (-0.11, 0.40)		0.13 (-0.16, 0.42)		0.13 (-0.22, 0.48)	
	Linear trend	0.05 (-0.002, 0.09)	0.060	0.05 (-0.008, 0.10)	0.098	0.06 (-0.003, 0.12)	0.061
		Unadjusted (n = 2907)		Adjusted[1] (n = 2325)		Adjusted[2] (n = 1694)	
Fathers	None	(ref)	0.101[a]	(ref)	0.186[a]	(ref)	0.128[a]
	1-2 days	-0.09 (-0.35, 0.16)		-0.16 (-0.44, 0.12)		-0.11 (-0.44, 0.22)	
	3-4 days	0.15 (-0.11, 0.40)		0.12 (-0.16, 0.40)		0.16 (0.17, 0.49)	
	5-10 days	-0.01 (-0.26, 0.23)		-0.04 (-0.31, 0.23)		-0.04 (-0.35, 0.28)	
	>10 days	0.19 (-0.06, 0.45)		0.11 (-0.17, 0.38)		0.23 (-0.09, 0.56)	
	Linear trend	0.05 (-0.008, 0.10)	0.096	0.04 (-0.02, 0.10)	0.242	0.06 (-0.01, 0.13)	0.121

Model [1]: socioeconomic position, income, home ownership, marital status, maternal education, gender, parity. Model [2]: socioeconomic position, income, home ownership, marital status, maternal education, gender, parity, maternal tobacco use during 1-3 months of pregnancy, maternal illicit drug use during 1-3 months of pregnancy, maternal depression 18 weeks gestation, maternal PRS for major depressive disorder [b]Omnibus p-value

Appendix 4.7: Associations between maternal and partner binge drinking and offspring total problems (SDQ) at age 17

		Unadjusted (n = 4888)		Adjusted[1] (n = 3761)		Adjusted[2] (n = 2674)	
		Coef (CI)	P	Coef (CI)	P	Coef (CI)	P
Mothers	None	(ref)	0.638[a]	(ref)	0.731[a]	(ref)	0.390[a]
	1-2 days	0.18 (-0.15, 0.51)		0.16 (-0.20, 0.53)		0.07 (-0.35, 0.50)	
	3-4 days	0.25 (-0.15, 0.66)		0.25 (-0.20, 0.70)		0.52 (-0.03, 1.06)	
	5-10 days	-0.007 (-0.45, 0.44)		-0.02 (-0.52, 0.48)		0.31 (-0.30, 0.91)	
	>10 days	0.24 (-0.34, 0.82)		0.26 (-0.39, 0.92)		0.27 (-0.53, 1.06)	
	Linear trend	0.04 (-0.06, 0.15)	0.432	0.04 (-0.08, 0.16)	0.483	0.11 (-0.03, 0.26)	0.126
		Unadjusted (n = 2889)		Adjusted[1] (n = 2311)		Adjusted[2] (n = 1685)	
Fathers	None	(ref)	0.087[a]	(ref)	0.082[a]	(ref)	0.225[a]
	1-2 days	-0.43 (-1.01, 0.14)		-0.62 (-1.25, 0.007)		-0.40 (-1.14, 0.34)	
	3-4 days	-0.35 (-0.92, 0.22)		-0.36 (-0.99, 0.27)		-0.27 (-1.02, 0.47)	
	5-10 days	-0.76 (-1.31, -0.22)		-0.82 (-1.42, -0.22)		-0.77 (-1.48, -0.06)	
	>10 days	-0.32 (-0.89, 0.24)		-0.39 (-1.01, 0.23)		-0.20 (-0.94, 0.53)	
	Linear trend	-0.09 (-0.22, 0.03)	0.134	-0.09 (-0.22, 0.05)	0.206	-0.07 (-0.23, 0.09)	0.392

Model [1]: socioeconomic position, income, home ownership, marital status, maternal education, gender, parity. Model [2]: socioeconomic position, income, home ownership, marital status, maternal education, gender, parity, maternal tobacco use during 1-3 months of pregnancy, maternal illicit drug use during 1-3 months of pregnancy, maternal depression 18 weeks gestation, maternal PRS for major depressive disorder [b]Omnibus p-value

Appendix 4.8: Associations between maternal binge drinking at 5 years and emotional problems (SDQ) at age 17

	Unadjusted (n = 4920)		Adjusted[1] (n = 3782)		Adjusted[2] (n = 2687)	
	Coef (CI)	p	Coef (CI)	p	Coef (CI)	p
None	(ref)	0.513[a]	(ref)	0.465[a]	(ref)	0.826[a]
1-2 days	0.03 (-0.10, 0.15)		0.06 (-0.08, 0.20)		0.01 (-0.15, 0.18)	
					0.02	
3-4 days	0.03 (-0.13, 0.19)		0.05 (-0.13, 0.22)		0.12 (-0.09, 0.32)	
5-10 days	-0.13 (-0.30, 0.05)		-0.12 (-0.31, 0.07)		-0.02 (-0.25, 0.21)	
>10 days	0.05 (-0.18, 0.27)		0.07 (-0.18, 0.32)		-0.03 (-0.33, 0.27)	
Linear trend	-0.01 (-0.05, 0.03)	0.648	1.01 (0.95, 1.06)	0.830	0.003 (-.05, 0.06)	0.904

Model [1]: socioeconomic position, income, home ownership, marital status, maternal education, gender, parity. Model [2]: socioeconomic position, income, home ownership, marital status, maternal education, gender, parity, maternal tobacco use during 1-3 months of pregnancy, maternal illicit drug use during 1-3 months of pregnancy, maternal depression 18 weeks gestation, maternal PRS for major depressive disorder. [b]Omnibus p-value

Appendix 4.9: Associations between maternal and partner alcohol frequency at 5 years and offspring depression (CIS-R) at age 18, imputed

		Unadjusted		Adjusted[1]		Adjusted[2]	
	(n = 11575)	OR (CI)	P	OR (CI)	P	OR (CI)	P
Mothers	None	(ref)	0.499[a]	(ref)	0.723[a]	(ref)	0.725[a]
	1-2 days	1.39 (0.86, 2.25)		1.37 (0.84, 2.23)		1.37 (0.84, 2.23)	
	3-4 days	1.40 (0.86, 2.29)		1.33 (0.81, 2.20)		1.33 (0.81, 2.20)	
	5-10 days	1.53 (0.89, 2.63)		1.40 (0.80, 2.44)		1.39 (0.79, 2.43)	
	>10 days	2.00 (0.83, 4.82)		1.69 (0.69, 4.12)		1.69 (0.69, 4.13)	
	Linear trend	1.10 (0.97, 1.25)	0.136	1.06 (0.93, 1.22)	0.368	1.06 (0.93, 1.22)	0.377
		Unadjusted		Adjusted[1]		Adjusted[2]	
Partners	None	(ref)	0.755[a]	(ref)	0.718[a]	(ref)	0.717[a]
	1-2 days	1.66 (0.66, 4.13)		1.63 (0.65, 4.08)		1.62 (0.65, 4.07)	
	3-4 days	1.46 (0.58, 3.68)		1.41 (0.55, 3.59)		1.41 (0.55, 3.58)	
	5-10 days	1.35 (0.52, 3.51)		1.27 (0.48, 3.37)		1.27 (0.48, 3.36)	
	>10 days	1.50 (0.54, 4.20)		1.36 (0.48, 3.90)		1.35 (0.47, 3.87)	
	Linear trend	0.98 (0.85, 1.13)	0.782	0.96 (0.82, 1.12)	0.589	0.96 (0.82, 1.12)	0.579

Model [1]: socioeconomic position, income, home ownership, marital status, maternal education, gender, parity. Model [2]: socioeconomic position, income, home ownership, marital status, maternal education, gender, parity, maternal tobacco use during 1-3 months of pregnancy, maternal illicit drug use during 1-3 months of pregnancy, maternal depression 18 weeks gestation, maternal PRS for major depressive disorder. [a]Wald test

Appendix 4.10: Associations between maternal and partner binge drinking at 5 years and offspring depression (CIS-R) at age 18, imputed

		Unadjusted		Adjusted[1]		Adjusted[2]	
	(n = 11575)	OR (CI)	P	OR (CI)	P	OR (CI)	P
Mothers	None	(ref)	0.795[a]	(ref)	0.754[a]	(ref)	0.759[a]
	1-2 days	1.18 (0.88, 1.58)		1.17 (0.87, 1.56)		1.17 (0.87, 1.56)	
	3-4 days	0.96 (0.66, 1.40)		0.91 (0.62, 1.33)		0.91 (0.62, 1.33)	
	5-10 days	1.05 (0.71, 1.56)		0.99 (0.67, 1.47)		0.99 (0.67, 1.47)	
	>10 days	1.10 (0.68, 1.79)		1.03 (0.63, 1.47)		1.03 (0.63, 1.68)	
	Linear trend	1.01 (0.92, 1.11)	0.820	0.99 (0.90, 1.09)	0.866	0.99 (0.90, 1.09)	0.862
		Unadjusted		Adjusted[1]		Adjusted[2]	
Partners	None	(ref)	0.621[a]	(ref)	0.603[a]	(ref)	0.605[a]
	1-2 days	1.40 (0.88, 2.23)		1.36 (0.85, 2.17)		1.36 (0.85, 2.16)	
	3-4 days	1.21 (0.75, 1.96)		1.20 (0.74, 1.95)		1.20 (0.74, 1.95)	
	5-10 days	1.22 (0.78, 1.91)		1.20 (0.76, 1.89)		1.20 (0.76, 1.89)	
	>10 days	1.06 (0.64, 1.77)		0.99 (0.59, 0.94)		0.99 (0.59, 1.67)	
	Linear trend	0.99 (0.90, 1.10)	0.864	0.98 (0.88, 1.09)	0.698	0.98 (0.88, 1.09)	0.695

Model [1]: socioeconomic position, income, home ownership, marital status, maternal education, gender, parity. Model [2]: socioeconomic position, income, home ownership, marital status, maternal education, gender, parity, maternal tobacco use during 1-3 months of pregnancy, maternal illicit drug use during 1-3 months of pregnancy, maternal depression 18 weeks gestation, maternal PRS for major depressive disorder [a]Wald test

Appendix 4.11: Associations between maternal and partner alcohol frequency at 5 years and offspring conduct problems (SDQ) at age 17, imputed

		Unadjusted		Adjusted[1]		Adjusted[2]	
	(n = 11575)	Coef (CI)	P	Coef (CI)	P	Coef (CI)	P
Mothers	None	(ref)	0.470[a]	(ref)	0.855[a]	(ref)	0.861[a]
	1-2 days	0.002 (-0.13, 0.13)		0.06 (-0.08, 0.19)		0.05 (-0.08, 0.19)	
	3-4 days	-0.07 (-0.21, 0.08)		0.04 (-0.10, 0.19)		0.04 (-0.10, 0.19)	
	5-10 days	-0.07 (-0.23, 0.10)		0.07 (-0.09, 0.24)		0.07 (-0.09, 0.23)	
	>10 days	-0.11 (-0.43, 0.21)		-0.03 (-0.35, 0.28)		-0.03 (-0.35, 0.28)	
	Linear trend	-0.03 (-0.08, 0.01)	0.151	0.008 (-0.04, 0.05)	0.732	0.007 (-0.04, 0.05)	0.752
		Unadjusted		Adjusted[1]		Adjusted[2]	
Partners	None	(ref)	0.011[a]	(ref)	0.362[a]	(ref)	0.349[a]
	1-2 days	-0.06 (-0.26, 0.14)		-0.01 (-0.21, 0.19)		-0.01 (-0.21, 0.18)	
	3-4 days	-0.17 (-0.39, 0.04)		-0.08 (-0.29, 0.13)		-0.08 (-0.29, 0.13)	
	5-10 days	-0.19 (-0.42, 0.04)		-0.07 (-0.29, 0.16)		-0.07 (-0.30, 0.16)	
	>10 days	-0.28 (-0.54, -0.03)		-0.16 (-0.41, 0.09)		-0.16 (-0.41, 0.09)	
	Linear trend	-0.07 (-0.12, -0.02)	0.006	-0.04 (-0.08, 0.01)	0.148	-0.04 (-0.08, 0.12)	0.142

Model [1]: socioeconomic position, income, home ownership, marital status, maternal education, gender, parity. Model [2]: socioeconomic position, income, home ownership, marital status, maternal education, gender, parity, maternal tobacco use during 1-3 months of pregnancy, maternal illicit drug use during 1-3 months of pregnancy, maternal depression 18 weeks gestation, maternal PRS for major depressive disorder. [a]Wald test

Appendix 4.12: Associations between maternal and partner binge drinking at 5 years and offspring conduct problems (SDQ) at age 17, imputed

		Unadjusted		Adjusted[1]		Adjusted[2]	
	(n = 11575)	Coef (CI)	P	Coef (CI)	P	Coef (CI)	P
Mothers	None	(ref)	0.169[a]	(ref)	0.207[a]	(ref)	0.209[a]
	1-2 days	0.08 (-0.01, 0.17)		0.09 (-0.0004, 0.18)		0.09 (-0.0007, 0.18)	
	3-4 days	0.12 (0.006, 0.24)		0.12 (-0.0007, 0.24)		0.12 (-0.0007, 0.24)	
	5-10 days	0.09 (-0.03, 0.22)		0.09 (-0.04, 0.21)		0.09 (-0.04, 0.21)	
	>10 days	0.11 (-0.07, 0.28)		0.08 (-0.10, 0.26)		0.08 (-0.10, 0.25)	
	Linear trend	0.03 (-0.001, 0.07)	0.057	0.03 (-0.006, 0.06)	0.113	0.03 (-0.007, 0.06)	0.114
				Adjusted[1]		Adjusted[2]	
Partners	None		0.980[a]	(ref)	0.928[a]	(ref)	0.930[a]
	1-2 days	-0.01 (-0.14, 0.12)		0.01 (-0.11, 0.13)		0.01 (-0.11, 0.13)	
	3-4 days	0.007 (-0.11, 0.13)		0.04 (-0.08, 0.16)		0.04 (-0.08, 0.16)	
	5-10 days	-0.01 (-0.14, 0.11)		0.20 (-0.11, 0.15)		0.02 (-0.11, 0.15)	
	>10 days	0.02 (-0.12, 0.16)		0.05 (-0.09, 0.19)		0.05 (-0.09, 0.19)	
	Linear trend	0.003 (-0.03, 0.03)	0.835	0.01 (-0.02, 0.04)	0.517	0.01 (-0.02, 0.04)	0.521

Model [1]: socioeconomic position, income, home ownership, marital status, maternal education, gender, parity. Model [2]: socioeconomic position, income, home ownership, marital status, maternal education, gender, parity, maternal tobacco use during 1-3 months of pregnancy, maternal illicit drug use during 1-3 months of pregnancy, maternal depression 18 weeks gestation, maternal PRS for major depressive disorder. [a]Wald test

Appendix 4.13: Associations between maternal and partner alcohol frequency at 5 years and offspring hyperactivity (SDQ) at age 17, imputed

		Unadjusted		Adjusted[1]		Adjusted[2]	
	(n = 11575)	Coef (CI)	P	Coef (CI)	P	Coef (CI)	P
Mothers	None	(ref)	0.016[a]	(ref)	0.267[a]	(ref)	0.265[a]
	1-2 days	-0.23 (-0.43, -0.03)		-0.15 (-0.35, 0.05)		-0.15 (-0.35, 0.04)	
	3-4 days	-0.34 (-0.55, -0.12)		-0.16 (-0.37, 0.06)		-0.16 (-0.37, 0.06)	
	5-10 days	-0.27 (-0.51, -0.03)		-0.03 (-0.27, 0.21)		-0.03 (-0.28, 0.21)	
	>10 days	-0.45 (-0.89, 0.003)		-0.31 (-0.76, 0.15)		-0.31 (-0.76, 0.14)	
	Linear trend	-0.08 (-0.15, -0.01)	0.017	-0.008 (-0.07, 0.06)	0.817	-0.008 (-0.08, 0.06)	0.803
		Unadjusted		Adjusted[1]		Adjusted[2]	
Partners	None	(ref)	0.059[a]	(ref)	0.800[a]	(ref)	0.795[a]
	1-2 days	-0.19 (-0.48, 0.11)		-0.12 (-0.41, 0.17)		-0.12 (-0.41, 0.17)	
	3-4 days	-0.31 (-0.62, -0.004)		-0.17 (-0.47, 0.14)		-0.17 (-0.48, 0.14)	
	5-10 days	-0.37 (-0.70, -0.05)		-0.18 (-0.51, 0.15)		-0.18 (-0.51, 0.15)	
	>10 days	-0.36 (-0.74, 0.03)		-0.15 (-0.54, 0.24)		-0.15 (-0.55, 0.24)	
	Linear trend	-0.08 (-0.16, -0.01)	0.026	-0.03 (-0.10, 0.05)	0.451	-0.03 (-0.10, 0.05)	0.444

Model [1]: socioeconomic position, income, home ownership, marital status, maternal education, gender, parity. Model [2]: socioeconomic position, income, home ownership, marital status, maternal education, gender, parity, maternal tobacco use during 1-3 months of pregnancy, maternal illicit drug use during 1-3 months of pregnancy, maternal depression 18 weeks gestation, maternal PRS for major depressive disorder. [a]Wald test

Appendix 4.14: Associations between maternal and partner binge drinking at 5 years and offspring hyperactivity (SDQ) at age 17, imputed

		Unadjusted		Adjusted[1]		Adjusted[2]	
	(n = 11575)	Coef (CI)	P	Coef (CI)	P	Coef (CI)	P
Mothers	None	(ref)	0.264[a]	(ref)	0.409[a]	(ref)	0.409[a]
	1-2 days	0.08 (-0.06, 0.21)		0.08 (-0.05, 0.21)		0.08 (-0.05, 0.21)	
	3-4 days	0.18 (0.01, 0.35)		0.16 (-0.005, 0.33)		0.16 (-0.004, 0.33)	
	5-10 days	0.07 (-0.12, 0.26)		0.04 (-0.15, 0.230		0.04 (-0.15, 0.23)	
	>10 days	0.15 (-0.10, 0.39)		0.07 (-0.18, 0.31)		0.07 (-0.18, 0.31)	
	Linear trend	0.04 (-0.01, 0.09)	0.109	0.02 (-0.02, 0.07)	0.328	0.02 (-0.02, 0.07)	0.331
		Unadjusted		Adjusted[1]		Adjusted[2]	
Partners	None	(ref)	0.542[a]	(ref)	0.645[a]	(ref)	0.645[a]
	1-2 days	-0.11 (-0.29, 0.08)		-0.05 (-0.24, 0.13)		-0.05 (-0.24, 0.13)	
	3-4 days	-0.01 (-0.18, 0.16)		0.03 (-0.14, 0.21)		0.03 (-0.14, 0.21)	
	5-10 days	-0.03 (-0.23, 0.17)		0.005 (-0.20, 0.20)		0.004 (-0.20, 0.20)	
	>10 days	0.06 (-0.15, 0.26)		0.09 (-0.12, 0.30)		0.09 (-0.12, 0.30)	
	Linear trend	0.02 (-0.03, 0.07)	0.432	0.02 (-0.03, 0.07)	0.348	0.02 (-0.03, 0.07)	0.350

Model [1]: socioeconomic position, income, home ownership, marital status, maternal education, gender, parity. Model [2]: socioeconomic position, income, home ownership, marital status, maternal education, gender, parity, maternal tobacco use during 1-3 months of pregnancy, maternal illicit drug use during 1-3 months of pregnancy, maternal depression 18 weeks gestation, maternal PRS for major depressive disorder [a]Wald test

Appendix 4.15: Associations between maternal and partner alcohol frequency at 5 years and offspring total problems (SDQ) at age 17, imputed

		Unadjusted		Adjusted[1]		Adjusted[2]	
	(n = 11575)	Coef (CI)	P	Coef (CI)	P	Coef (CI)	P
Mothers	None	(ref)	0.0002[a]	(ref)	0.144[a]	(ref)	0.140[a]
	1-2 days	-0.57 (-1.00, -0.14)		-0.36 (-0.80, 0.07)		-0.37 (-0.80, 0.07)	
	3-4 days	-0.98 (-0.144, -0.51)		-0.53 (-0.99, -0.06)		-0.53 (1.00, -0.06)	
	5-10 days	-0.83 (-1.36, -0.30)		-0.24 (-0.78, 0.30)		-0.24 (-0.78, 0.30)	
	>10 days	-1.04 (-2.09, 0.005)		-0.73 (-1.78, 0.32)		-0.73 (-1.78, 0.31)	
	Linear trend	-0.26 (-0.41, -0.11)	0.001	-0.08 (-0.23, 0.07)	0.279	-0.08 (-0.24, 0.07)	0.270
		Unadjusted		Adjusted[1]		Adjusted[2]	
Partners	None	(ref)	0.0001[a]	(ref)	0.144[a]	(ref)	0.140[a]
	1-2 days	-0.39 (-1.07, 0.29)		-0.21 (-0.89, 0.46)		-0.21 (-0.89, 0.46)	
	3-4 days	-0.93 (-1.66, -0.20)		-0.53 (-1.25, 0.19)		-0.53 (-1.26, 0.19)	
	5-10 days	-1.14 (-1.91, -0.37)		-0.59 (-1.36, 1.19)		-0.59 (-1.37, 0.19)	
	>10 days	-1.24 (-2.14, -0.33)		-0.68 (-1.59, 0.22)		-0.69 (-1.60, 0.21)	
	Linear trend	-0.32 (-0.49, -0.15)	<0.001	-0.17 (-0.34, 0.002)	0.052	-0.17 (-0.34,0.001)	0.051

Model [1]: socioeconomic position, income, home ownership, marital status, maternal education, gender, parity. Model [2]: socioeconomic position, income, home ownership, marital status, maternal education, gender, parity, maternal tobacco use during 1-3 months of pregnancy, maternal illicit drug use during 1-3 months of pregnancy, maternal depression 18 weeks gestation, maternal PRS for major depressive disorder. [a]Wald test

Appendix 4.16: Associations between maternal and partner binge drinking at 5 years and offspring total problems (SDQ), at age 17 imputed

		Unadjusted		Adjusted[1]		Adjusted[2]	
	(n = 11575)	Coef (CI)	P	Coef (CI)	P	Coef (CI)	P
Mothers	None	(ref)	0.892[a]	(ref)	0.914[a]	(ref)	0.916[a]
	1-2 days	0.09 (-0.22, 0.40)		0.13 (-0.17, 0.43)		0.13 (-0.18, 0.43)	
	3-4 days	0.12 (-0.25, 0.49)		0.10 (-0.27, 0.47)		0.10 (-0.27, 0.47)	
	5-10 days	-0.03 (-0.45, 0.40)		-0.04 (-0.46, 0.38)		-0.04 (-0.46, 0.38)	
	>10 days	0.21 (-0.35, 0.78)		0.07 (-0.50, 0.64)		0.07 (-0.50, 0.64)	
	Linear trend	0.03 (-0.08, 0.14)	0.619	0.008 (-0.10, 0.12)	0.890	0.08 (-0.10, 0.12)	0.894
		Unadjusted		Adjusted[1]		Adjusted[2]	
Partners	None	(ref)	0.147[a]	(ref)	0.523[a]	(ref)	0.519[a]
	1-2 days	-0.33 (-0.77, 0.11)		-0.23 (-0.66, 0.20)		-0.23 (-0.66, 0.20)	
	3-4 days	-0.39 (-0.80, 0.02)		-0.25 (-0.65, 0.16)		-0.25 (-0.65, 0.16)	
	5-10 days	-0.53 (-0.99, -0.07)		-0.37, -0.81, 0.08)		-0.37 (-0.81, 0.08)	
	>10 days	-0.46 (-0.95, 0.03)		-0.30 (-0.79, 0.18)		-0.31 (-0.79, 0.18)	
	Linear trend	-0.11 (-0.23, 0.006)	0.063	-0.07 (-0.19, 0.04)	0.213	-0.07 (-0.19, 0.04)	0.212

Model [1]: socioeconomic position, income, home ownership, marital status, maternal education, gender, parity. Model [2]: socioeconomic position, income, home ownership, marital status, maternal education, gender, parity, maternal tobacco use during 1-3 months of pregnancy, maternal illicit drug use during 1-3 months of pregnancy, maternal depression 18 weeks gestation, maternal PRS for major depressive disorder [a]Wald test

Appendix 4.17: Associations between maternal and partner alcohol frequency at 5 years and offspring emotional problems (SDQ) at age 17, imputed

		Unadjusted		Adjusted[1]		Adjusted[2]	
	(n = 11575)	Coef (CI)	P	Coef (CI)	P	Coef (CI)	P
Mothers	None	(ref)	0.332[a]	(ref)	0.711[a]	(ref)	0.709[a]
	1-2 days	-0.09 (-0.28, 0.10)		-0.05 (-0.24, 0.14)		-0.05 (-0.24, 0.14)	
	3-4 days	-0.17 (-0.36, 0.02)		-0.09 (-0.28, 0.10)		-0.09 (-0.28, 0.10)	
	5-10 days	-0.12 (-0.33, 0.09)		-0.02 (-0.24, 0.20)		-0.02 (-0.24, 0.19)	
	>10 days	-0.22 (-0.62, 0.18)		-0.21 (-0.60, 0.17)		-0.21 (-0.60, 0.17)	
	Linear trend	-0.05 (-0.10, 0.10)	0.107	-0.02 (-0.07, 0.04)	0.549	-0.02 (-0.07, 0.04)	0.543
		Unadjusted		Adjusted[1]		Adjusted[2]	
Partners	None	(ref)	0.300[a]	(ref)	0.949[a]	(ref)	0.948[a]
	1-2 days	-0.03 (-0.31, 0.24)		0.005 (-0.27, 0.28)		0.005 (-0.27, 0.28)	
	3-4 days	-0.15 (-0.44, 0.15)		-0.05 (-0.34, 0.24)		-0.05 (-0.34, 0.24)	
	5-10 days	-0.17 (-0.44, 0.15)		-0.03 (-0.35, 0.30)		-0.03 (-0.35, 0.30)	
	>10 days	-0.17 (-0.49, 0.15)		-0.04 (-0.41, 0.32)		-0.05 (-0.41, 0.32)	
	Linear trend	-0.05 (-0.12, 0.14)	0.126	-0.01 (-0.08, 0.06)	0.713	-0.01 (-0.08, 0.06)	0.708

Model [1]: socioeconomic position, income, home ownership, marital status, maternal education, gender, parity. Model [2]: socioeconomic position, income, home ownership, marital status, maternal education, gender, parity, maternal tobacco use during 1-3 months of pregnancy, maternal illicit drug use during 1-3 months of pregnancy, maternal depression 18 weeks gestation, maternal PRS for major depressive disorder. [a]Wald test

Appendix 4.18: Associations between maternal and partner binge drinking at 5 years and offspring emotional problems (SDQ) at age 17, imputed

		Unadjusted		Adjusted[1]		Adjusted[2]	
	(n = 11575)	Coef (CI)	P	Coef (CI)	P	Coef (CI)	P
Mothers	None	(ref)	0.665[a]	(ref)	0.677[a]	(ref)	0.677[a]
	1-2 days	-0.008 (-0.13, 0.11)		0.007 (-0.11, 0.12)		0.007 (-0.11, 0.12)	
	3-4 days	-0.007 (-0.15, 0.14)		-0.02 (-0.16, 0.12)		-0.02 (-0.16, 0.14)	
	5-10 days	-0.11 (-0.27, 0.06)		-0.11 (-0.27, 0.05)		-0.11 (-0.27, 0.05)	
	>10 days	-0.05 (-0.15, 0.26)		0.01 (-0.18, 0.21)		0.01 (-0.18, 0.21)	
	Linear trend	-0.008 (-0.05, 0.03)	0.694	1.02 (0.98, 1.06)	0.328	-0.01 (-0.06, 0.03)	0.480
		Unadjusted		Adjusted[1]		Adjusted[2]	
Partners	None	(ref)	0.033[a]	(ref)	0.200[a]	(ref)	0.199[a]
	1-2 days	-0.06 (0.23, 0.11)		-0.05 (-0.22, 0.11)		-0.05 (-0.22, 0.11)	
	3-4 days	-0.18 (-0.35, -0.02)		-0.14 (-0.30, 0.02)		-0.14 (-0.30, 0.02)	
	5-10 days	-0.21 (-0.37, -0.04)		-0.15 (-0.32, 0.01)		-0.15 (-0.32, 0.01)	
	>10 days	-0.21 (-0.40, -0.02)		-0.16 (-0.35, 0.02)		-0.16 (-0.35, 0.02)	
	Linear trend	-0.06 (-0.10, -0.01)	0.012	0.99 (0.95, 1.03)	0.549	-0.04 (-0.09, 0.001)	0.057

Model [1]: socioeconomic position, income, home ownership, marital status, maternal education, gender, parity. Model [2]: socioeconomic position, income, home ownership, marital status, maternal education, gender, parity, maternal tobacco use during 1-3 months of pregnancy, maternal illicit drug use during 1-3 months of pregnancy, maternal depression 18 weeks gestation, maternal PRS for major depressive disorder. [a]Wald test

Appendix 5.1

SNPs used to make alcohol PRS
rs705687
rs58107686
rs12088813
rs5024204
rs10753661
rs28680958
rs823114
rs77165542
rs1260326
rs2178197
rs13383034
rs1004787
rs13032049
rs828867
rs11692435
rs13024996
rs72859280
rs56337305
rs13094887
rs62250685
rs74664784
rs13066454
rs9838144
rs2011092
rs60654199
rs6787172
rs3748034
rs7682824
rs11940694
rs35538052
rs4501255
rs12499107
rs144198753
rs1154414
rs1229984
rs10028756
rs561222871
rs36052336
rs2165670
rs17029090
rs79139602
rs4699791
rs13107325
rs4690727

rs10004020
rs12651313
rs4916723
rs12655091
rs55872084
rs11739827
rs10085696
rs6460047
rs10236149
rs35034355
rs6951574
rs13250583
rs1217091
rs28601761
rs55932213
rs10978550
rs7074871
rs17665139
rs7950166
rs11030084
rs56030824
rs10750025
rs1713676
rs4938230
rs682011
rs12795042
rs10876188
rs3809162
rs10506274
rs4842786
rs500321
rs1123285
rs2180870
rs28929474
rs11625650
rs2472297
rs12907323
rs2764771
rs17177078
rs378421
rs113443718
rs62044525
rs7185555
rs79616692
rs1104608
rs4548913

rs3803800
rs2854334
rs2532276
rs10438820
rs9950000
rs4092465
rs281379
rs4815364
rs9607814

Appendix 5.2: Permutation analyses of maternal pregnancy phenotypes and maternal PRS for alcohol consumption

Phenotype	Tobs	prop	lowerCI	upperCI
Depression 32 weeks	0.092430986	0.016000001	0.009172319	0.025853248
Neuroticism	0.16413787	0.123000003	0.103276908	0.144972235
Depression 18 weeks	0.060914848	0.126000002	0.106056832	0.14817372
Smoked 1-3 months	0.047895133	0.128999993	0.10884057	0.151371419
Never smoked	-0.042689145	0.129999995	0.109769315	0.152436495
Life events	0.012600807	0.158000007	0.135926515	0.182107598
Reduced cigarettes	0.049414	0.166	0.143449	0.190537
Education	0.023924	0.191	0.167075	0.216759
Smoked cannabis 1-3 months	0.098661	0.222	0.196591	0.249057
Increased cigarettes	0.351009	0.242	0.215746	0.269782
Social class	-0.01878	0.267	0.2398	0.295579
Vomited in pregnancy	-0.03171	0.279	0.251386	0.307921
Daily caffeine intake	1.407763	0.35	0.320416	0.38047
No change in caffeine	-0.02003	0.374	0.343919	0.404825
Hypersensitivity to rejection	0.177089	0.419	0.388197	0.450282
Sleep initiation	0.008048	0.496	0.464562	0.527462
Increased caffeine	-0.02893	0.514	0.48252	0.545397
Image perception	0.035877	0.527	0.495515	0.558326
Craved more caffeine	0.022349	0.564	0.532615	0.595008
Physical activity perception	0.005978	0.586	0.554756	0.616736
Ever drank caffeine	-0.01337	0.606	0.574939	0.636435
Reduced caffeine	-0.01024	0.663	0.632756	0.69228
No change in cigarettes	0.016403	0.816	0.790566	0.839558

Illicit drugs in pregnancy	-0.04401	0.819	0.793709	0.842396
Reaction to becoming a parent	-0.00236	0.839	0.814729	0.861255
Image perception change	-0.00991	0.84	0.815783	0.862195
Stopped smoking	0.010352	0.853	0.829516	0.874383
Craved more cigarettes	0.019798	0.909	0.889449	0.926101
Average income	-0.0003	0.985	0.97538	0.991581

Appendix 5.3: Permutation analyses of child phenotypes and child PRS for alcohol consumption

Phenotype	Tobs	prop	lowerCI	upperCI
Sleep duration	-0.01964	0.223	0.197547	0.250095
Handedness	-0.08106	0.229	0.203286	0.25632
Sleep maintenance age 7	-0.0466	0.243	0.216706	0.270816
Frequency smokes cannabis	-0.07175	0.312	0.283373	0.341738
Ever smoked age 23	0.059983	0.317	0.288235	0.346847
Big 5 personality measure: Conscientiousness	-0.12798	0.393	0.362581	0.424051
Lifetime cigarettes smoked age 14	0.094084	0.409	0.378334	0.440203
Ever smoked cannabis	-0.05236	0.431	0.400049	0.462359
Specific phobia	0.132772	0.488	0.456593	0.519477
Lifetime cigarettes smoked age 18	0.070771	0.503	0.471541	0.534441
Eating disorder age 13	-0.22319	0.522	0.490588	0.553814
Daily caffeine intake age 13	0.57314	0.527	0.495515	0.558326
Big 5 personality measure: Emotional stability	-0.10867	0.529	0.497516	0.560313
Life events age 7	0.007643	0.548	0.51655	0.579166
Psychosis negative symptoms age 16	-0.0957	0.556	0.524578	0.587091
Oppositional defiant disorder age 15	-0.01115	0.564	0.532615	0.595008
ADHD	-0.01920	0.545	0.513542	0.576192
Daily caffeine intake age 8	0.2678	0.624	0.593149	0.654119

Exercise frequency	0.009444	0.643	0.612418	0.672737
Oppositional defiant disorder age 7	0.006577	0.655	0.624614	0.68447
Hyperactivity symptoms	-0.00523	0.683	0.653153	0.711765
Big 5 personality measure: Extraversion	0.06909	0.688	0.658262	0.716627
Depression age 18	-0.06117	0.688	0.658262	0.716627
Education (GCSE D-G)	-0.04583	0.693	0.663374	0.721485
BMI age 7	-0.01497	0.708	0.678736	0.736034
Age of first cigarette	-0.05119	0.725	0.69619	0.752479
Autism	-0.06842	0.731	0.702362	0.758271
Depression age 14	0.038703	0.737	0.708541	0.764057
Depression symptom score	0.042785	0.751	0.722983	0.777531
Eating disorder age 16	0.075539	0.759	0.731252	0.785214
Phobia symptom score	0.006163	0.761	0.733321	0.787133
Anxiety	-0.00605	0.783	0.756138	0.808185
Number of cigarettes smoked daily	-0.0026	0.795	0.768627	0.819623
Conduct disorder age 16	0.17025	0.619	0.588086	0.649211
Life events age 16	0.004338	0.807	0.78115	0.831028
Total behavioural difficulties age 7	0.023378	0.807	0.78115	0.831028
Number of cigarettes smoked weekly	0.002832	0.809	0.783241	0.832925
Total behavioural difficulties age 17	-0.02808	0.814	0.788472	0.837664
Emotional symptoms score	0.005791	0.829	0.804205	0.85184
Conduct disorder age 7	0.002783	0.83	0.805256	0.852783
Suicide attempt	-0.03096	0.841	0.816838	0.863134
Sleep maintenance age 15	0.005252	0.844	0.820003	0.865951
Big 5 personality measure: Intellect	-0.02634	0.859	0.835873	0.879988
Anxiety symptoms score	-0.00575	0.873	0.85076	0.893016
IQ age 15	-0.05847	0.876	0.85396	0.895797
Psychosis positive symptoms	-0.00174	0.884	0.862513	0.903194
Sleep duration	0.003974	0.905	0.885115	0.92246
Big 5 personality measure: Agreeableness	0.015139	0.918	0.899237	0.934254

Lifetime cigarettes smoked age 23	0.006595	0.921	0.902513	0.936959
Ever smoked	-0.00911	0.929	0.911284	0.944135
Depression age 10	0.003713	0.937	0.920112	0.951253
Age of first cigarette	0.002186	0.95	0.93461	0.962665
Ever smoked age 18	-0.00304	0.954	0.939116	0.966129
BMI age 17	-0.00573	0.955	0.940247	0.96699
Sleep initiation age 15	0.017167	0.959	0.944788	0.97042
Education (GCSE A-C)	0.004978	0.973	0.960919	0.982115
Psychosis positive symptoms age 18	0.000326	0.985	0.97538	0.991581
PTSD	-0.00015	0.987	0.977872	0.99306
IQ age 7	-0.00425	0.988	0.979132	0.993784
Psychosis positive symptoms	0.000681	0.99	0.981687	0.995194
Sleep initiation age 7	-0.00027	0.996	0.98979	0.998909

Appendix 5.4: Permutation analyses of intergenerational analyses. Child phenotypes and maternal PRS for alcohol consumption

Phenotype	Tobs	prop	lowerCI	upperCI
Daily caffeine intake age 8	0.777552	0.14	0.119078	0.163066
Big 5 personality measure: Intellect	-0.21082	0.154	0.132173	0.177885
Ever smoked age 23	0.062897	0.167	0.14439	0.191589
ADHD	0.0408546	0.237	0.2109495	0.2646085
Depression age 10	0.071943	0.251	0.224392	0.279083
Big 5 personality measure: Conscientiousness	-0.15893	0.283	0.255254	0.31203
Exercise frequency	0.020345	0.307	0.278515	0.336626
Depression age 14	0.107127	0.39	0.359631	0.421018
BMI age 7	-0.03377	0.392	0.361598	0.42304
Total behavioural difficulties age 7	0.071877	0.412	0.381292	0.443228
IQ age 15	0.314028	0.427	0.396096	0.458335
Sleep maintenance age 7	-0.02955	0.456	0.424799	0.487461
Ever smoked	0.043449	0.467	0.435714	0.498481
Age of first cigarette	-0.02602	0.471	0.439687	0.502484
Education (GCSE D-G)	-0.07612	0.48	0.448633	0.511485

BMI age 17	-0.08631	0.497	0.465559	0.528459
Lifetime cigarettes smoked age 14	0.075523	0.527	0.495515	0.558326
Big 5 personality measure: Extraversion	0.111626	0.531	0.499517	0.5623
Frequency smokes cannabis	-0.04767	0.556	0.524578	0.587091
Conduct disorder age 16	-0.002527	0.925	0.906892	0.940553
Hyperactivity symptoms	0.006829	0.57	0.538647	0.60094
Sleep initiation age 15	-0.25496	0.582	0.550726	0.61279
Big 5 personality measure: Agreeableness	-0.07116	0.589	0.55778	0.619694
Psychosis positive symptoms	0.027886	0.589	0.55778	0.619694
Education (GCSE A-C)	0.123912	0.595	0.563832	0.625607
Sleep maintenance age 15	0.011637	0.645	0.614449	0.674694
Psychosis positive symptoms age 18	-0.00647	0.66	0.629702	0.689353
Anxiety	-0.01192	0.664	0.633775	0.693256
Autism	0.078919	0.696	0.666444	0.724397
Depression age 18	-0.05644	0.72	0.691051	0.747647
Eating disorder age 16	0.093223	0.723	0.694134	0.750547
Lifetime cigarettes smoked age 18	-0.03711	0.734	0.705451	0.761164
Sleep initiation age 7	0.013582	0.741	0.712663	0.76791
Age of first cigarette	-0.04388	0.741	0.712663	0.76791
Ever smoked age 18	0.033043	0.746	0.717821	0.772723
Ever smoked cannabis	0.020343	0.75	0.72195	0.77657
Total behavioural difficulties age 17	0.034087	0.783	0.756138	0.808185
Psychosis negative symptoms age 16	0.047366	0.786	0.759257	0.811047
Oppositional defiant disorder age 7	-0.00339	0.804	0.778016	0.82818
Phobia symptom score	0.00484	0.817	0.791613	0.840504
Lifetime cigarettes smoked age 23	0.012947	0.819	0.793709	0.842396
Eating disorder age 13	0.076669	0.825956	0.800927	0.849036
Handedness	0.012338	0.844	0.820003	0.865951
Life events age 16	-0.00312	0.846	0.822115	0.867827
IQ age 7	0.068031	0.855	0.831634	0.876253

Specific phobia	0.035553	0.855	0.831634	0.876253
Sleep duration	-0.00311	0.856	0.832693	0.877187
Depression symptom score	0.018364	0.878	0.856096	0.897649
Emotional symptoms score	0.004412	0.885	0.863584	0.904117
Sleep duration	-0.00408	0.886	0.864656	0.905039
Psychosis positive symptoms	-0.00163	0.886	0.864656	0.905039
Anxiety symptoms score	0.00454	0.887	0.865728	0.90596
Conduct disorder age 7	0.008295	0.558	0.526587	0.589071
Daily caffeine intake age 13	-0.0987	0.905	0.885115	0.92246
Number of cigarettes smoked weekly	0.001097	0.921	0.902513	0.936959
Suicide attempt	0.01188	0.931	0.913485	0.945921
Big 5 personality measure: Emotional stability	0.012627	0.948	0.932365	0.960923
Number of cigarettes smoked daily	-0.00066	0.956	0.94138	0.967851
Life events age 7	0.000276	0.981	0.970488	0.988523
Oppositional defiant disorder age 15	0.000314	0.988	0.979132	0.993784
PTSD	0.000116	0.996	0.98979	0.998909

Appendix 6.1. Fit indices for sex invariant conduct problem classes

	BIC	Entropy
One group	49353.16	
Two group	43599.36	0.91
Three group	43301.74	0.81
Four group	43198.07	0.79

Appendices 6.2. Internal consistency of the parent reported SDQ scales by gender

Cronbach's α		Gender	
		Male	Female
SDQ parent report	*n* = 4384	*n* = 2303	*n* = 2303
Emotional symptoms	0.61	0.63	0.60
Conduct problems	0.51	0.55	0.44
Hyperactivity	0.78	0.79	0.75
Peer problems	0.49	0.50	0.47
Prosocial behaviour	0.63	0.64	0.59
Total difficulties score	0.77	0.79	0.74

Appendices 6.3. Validity between the SDQ and CBCL

	SDQ parent report					
CBCL scale	**Emotional**	**Conduct**	**Hyperactivity**	**Peer**	**Prosocial**	**Total**
Internalising	0.62	0.24	0.27	0.33	-0.09	0.51
Anxious depressed	0.59	0.23	0.25	0.29	-0.10	0.48
Withdrawn/depressed	0.43	0.27	0.19	0.48	-0.19	0.46
Somatic complaints	0.47	0.12	0.16	0.13	0.00	0.31
Externalising	0.36	0.60	0.47	0.38	-0.28	0.63
Rule-breaking	0.28	0.54	0.41	0.27	-0.23	0.52
Aggressive	0.36	0.58	0.47	0.39	-0.27	0.63
Social problems	0.43	0.36	0.34	0.47	-0.24	0.55
Thought problems	0.51	0.37	0.40	0.38	-0.17	0.59
Attention problems	0.35	0.47	0.75	0.31	-0.23	0.71
Total problems	0.52	0.51	0.56	0.42	-0.23	0.72